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BIOPHYSICAL CHARACTERIZATION OF REAGINIC AND BLOCKING SERA

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CAMPBELL, Cann, Friedman, and Brown[1, 2] applied the method of electrophoresis-convection to the study of the distribution of allergic antibodies among the proteins of reaginic sera. Their pioneering study indicated that reagins, or skin-sensitizing antibodies, were not characteristically associated with the γ-globulin, as previously supposed, but were associated instead with the α- and/or β-globulins in two of the sera studied. Subsequently, Loveless and Cann[3-5] utilized electrophoresis-convection in an extensive investigation of a number of sera containing reaginic and/or blocking antibodies. (The latter bind and inactivate the common antigen, thereby depriving the allergic antibody of this participant in the wheal-and-flare reaction.) From these investigations has emerged the fundamental fact that reagins and blocking antibodies are distinct physical entities. Whereas reagin appears to be associated chiefly with the β-globulin, the blocking antibody migrates electrophoretically with the γ-globulins. The purpose of the present article is to illustrate, with representative cases, the way in which the combined application of biophysics and immunology serves to differentiate two antibodies. Whereas the results for the blocking serum have been reported previously,[5] those for the reaginic serum are being published for the first time.

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METHODS

Electrophoresis-Convection.—The method of electrophoresis-convection was first proposed and applied to the fractionation of proteins in solution by Nielsen and Kirkwood.[6, 7] The procedure has since been used successfully on a number of protein systems.[8-11] Electrophoresis-convection is a mild physical method of fractionation which yields a large number of relatively homogeneous fractions from a protein mixture. The quantity of fraction procured is adequate to permit thorough biophysical and immunologic characterization. In addition to minimizing the risk of denaturation during fractionation, electrophoresis-convection possesses several advantages over other methods. Thus, for example, the proteins do not come into contact with supporting media, such as starch, which might react with the sample. Furthermore, starch usually contains extractable materials like dextrans and mold spores, both of which are common allergens. They might set up antigen-antibody reactions in the test subject's skin during passive transfer testing of the fractions and thereby reduce the responsiveness of the site to subsequent tests with the antigen being studied.

Electrophoresis-convection is based on the principle employed in the Clusius column. However, horizontal electrophoretic transport, rather than thermal diffusion, is responsible for the convective transport which occurs in the vertical channel. The scheme of fractionation may be described briefly as follows: Two reservoirs are connected by a vertical convection channel which is sufficiently narrow to insure laminar flow. The unit is then filled with the protein solution to be fractionated. Upon application of a horizontal electric field, differential transport of the mobile components takes place across the channel and a density gradient is built up where the moving molecules are stopped by the cellophane wall. Under the influence of gravity, the molecules descend into the lower compartment, thereby initiating convective circulation in the channel. Eventually, the top reservoir loses all its mobile components and is comprised exclusively of immobile constituents. Meanwhile, the lower compartment is greatly enriched in mobile molecules. To avoid contamination of the protein solution by electrolysis products, the walls of the convection channel are constructed of semipermeable membranes which are separated from the electrodes by buffer solution. The electric field across the channel is maintained by an electric current which is carried through the permeable membrane by the ions of the buffer electrolyte. The exterior buffer solution is replenished by a circulatory system at a rate sufficient to prevent electrolysis products from reaching the membranes. This assures a constant pH. The separatory operation is carried out in a constant-temperature cold room at 4° C., at a field strength of 1 to 4 volts per cubic centimeter, and each run requires from forty-eight to seventy-two hours.

The proteins in a mixture such as serum possess discrete mobility and isoelectric point spectra. The separation of such a mixture into its constituent proteins is accomplished by successive immobilization of the components at their respective isoelectric points and by transport of the mobile components

from the top reservoir to the bottom reservoir of the apparatus. Thus, γ-globulin, which is known to be heterogeneous, can be separated from the other constituents of human serum by several successive stages of operation at its mean isoelectric point. On the other hand, by operating at a pH displaced by a slight arbitrary amount from the mean isoelectric point of γ-globulin, a fraction of γ-globulin rather than the entire "family" of γ-globulins can be separated from the serum. Consequently, if successive stages of fractionation are carried out at different pH levels, a number of γ-globulin fractions possessing different mean electrophoretic mobilities can be prepared. Fractions rich in β-globulin can be similarly obtained. In the present study successive stages of fractionation were carried out at pH values which were lowered stepwise from 8.1 to 5.2 at intervals of about 0.5 pH unit. At one point (Stage Va, pH 6.0) the pH was not lowered for the next two or three stages (Vb, Ve) as a large supply of this reagin-enriched fraction was desired. Serum, which had been diluted with one volume of buffer and then dialyzed versus buffer, served as the starting material for the first stage. The protein solution removed from the top reservoir at the end of each stage was designated the "top" fraction. The solution removed from the bottom reservoir served as the starting material for the succeeding stage, the original volume being restored with the appropriate buffer. At the conclusion of each run, the "top" fraction was dialyzed against phosphate buffer at pH 7.0, passed through a bacterial filter, and stored without preservative at 4° C. until fractionation was completed. The bottom fraction of the final stage of fractionation was mixed with one volume of saturated ammonium sulfate at pH 7 to provide a globulin (BG) and an albumin (BA) fraction.

In the case of the reaginic serum discussed in this article, top VIa (obtained at pH 5.4) was refractionated in the electrophoresis-convection apparatus at pH 5.2 to give two new fractions (top and bottom VIb).

All yields were determined by Kjeldahl digestion of the fractions and by subsequent nesslerization. Every effort was made to avoid contamination throughout these experiments, and periodic cultures for bacteria were made. Contaminations were encountered occasionally, but were promptly interrupted by filtration.

Electrophoretic Analyses.—Electrophoretic analyses of the various fractions were carried out by the moving-boundary technique in the Perkin-Elmer Tiselius apparatus. Currents were determined by measuring the potential drop across a standard resistance, using a potentiometer. Analyses were carried out on solutions containing about 1 per cent protein in barbital buffer, pH 8.6 and ionic strength 0.1. Mobilities were calculated from the descending patterns in the conventional manner, and the areas under enlarged schlieren peaks were measured with a planimeter.

Immunologic Tests.— The several immunologic tests utilized in this study were of a controlled variety. Since they have been previously described in detail,[3-5] only brief reference need be made here. The *skin-sensitizing* activities of the various sera and of their fractions were appraised in two ways; tests

were repeated in several normal subjects on three or four different occasions, some sixty-four sites being tested in each experiment. The serum-dilution and the serum-neutralization, or absorption, techniques were the methods utilized. In the former, a series of twofold dilutions of a given fraction is set up in isotonic saline solution. With the latter, mixtures are prepared with one volume of sensitizing serum or fraction, one volume of allergen in doubling concentration, and one volume of either saline solution or normal serum. Each of these preparations is injected intradermally in a volume of 0.1 c.c. into the back of the normal subject selected for the experiment. Challenges with a standard volume and strength of allergen are made on the following day. That site which shows only a minimal wheal-and-flare response serves as the end point. The end point in the serum-dilution test reveals the greatest dilution of the fraction which can still transfer sensitiveness to normal skin. The end point of the neutralization test indicates the minimal quantity of allergen necessary to absorb out the sensitizing activity. Thus, with the absorption procedure, it is possible to measure reaginic activity in terms of the exact quantity of chemically standardized allergen required to negate the skin-sensitizing power of 1 mg. of serum protein in the fraction.

The *thermostable*, or *blocking antibody*, content of fractions was determined by a direct inhibition test. With this technique, the presence of excess antigen in a serum-antigen mixture is revealed by its capacity to provoke a whealing response in previously sensitized normal skin. If blocking antibody is present in the serum, more antigen must be included in the mixture than when the normal serum of a control subject has been mixed with the antigen. The difference between the two sets of end points expresses the antigen-binding power of the immune specimen in terms of units of antigen blocked per milligram of protein in the serum fraction. Our routine twofold dilutions of antigen are used in these tests.

Serum.—The cottonseed-reaginic serum is designated SEN 11/11/53. The donor of this serum is a middle-aged, asthmatic woman who is highly allergic to cottonseed by history, as well as by intracutaneous, conjunctival, serologic, and ingestion tests. She has never received therapeutic injections of seed extract. The insulin-resistant serum, BER 2/3/53, was donated by a diabetic woman in her middle sixties. At the time of venipuncture, control of her diabetes demanded no fewer than 900 to 1,100 units of regular insulin daily. Her serum inhibited the hypoglycemic activity of insulin for fasted mice and blocked the allergenic activity of insulin for sensitized human skin. Thus, as far as insulin was concerned, her serum was antihormonal and antiallergenic.

The antigens used in this study were the purified allergen, CS54R, described by Spies and associates,[12] and the crystalline pork and beef insulin of Squibb. The former was standardized on the basis of its protein nitrogen, one unit representing 0.01 meg. of nitrogen, while the latter was expressed in conventional hormonal units.

RESULTS

Reaginic Serum.—SEN 11/11/53 cottonseed-reaginic serum was carried through nine successive stages of fractionation by electrophoresis-convection. The results of the characterization of the various fractions will be summarized

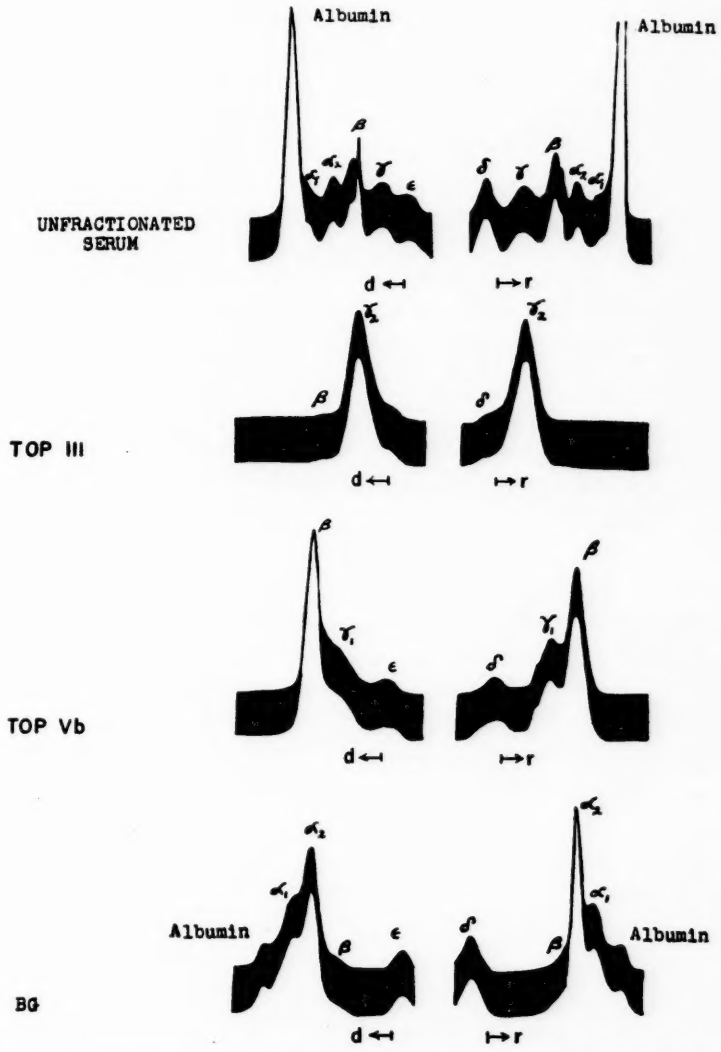

Fig. 1.—Electrophoretic patterns of several fractions of cottonseed-reaginic serum, SEN 11/11/53.

by means of several figures and tables. For example, the electrophoretic patterns of the original serum and of several fractions are presented in Fig. 1, while the electrophoretic compositions are given in Table I. To ascertain whether sensitizing activity was associated principally with one or another of the electrophoretic components of the serum, the reaginic activities of the

TABLE I. ELECTROPHORETIC COMPOSITION OF SEN 11/11/53 COTTONSEED-REAGINIC SERUM FRACTIONS

FRACTION	ELECTROPHORETIC COMPOSITION* (% OF FRACTION)						MOBILITY OF γ_2-GLOBULIN – 10^5 × μ CM.^{2} SEC.$^{-1}$ VOLT^{-1}
	ALBUMIN	α_1-GLOBULIN	α_2-GLOBULIN	β-GLOBULIN	γ_1-GLOBULIN†	γ_2-GLOBULIN	
Unfractionated serum	51	6	11	19		13	1.10
I	1			2		97	0.93
II				3		97	1.26
III				1		99	1.49
IV				9		91	1.63
Va				66		34	1.72
Vb				74	26		
Vc	2	5		77	16		
VIa	8	17	43	32	‡		
VIb		6	42	52			
Bottom VIb	27	11	41	21			
VII	21	18	40	21			
BG	7	34	55	4			
BA	89	6	3	2			

*Composition of descending patterns in barbital buffer, pH 8.6 and ionic strength 0.1.
†γ-globulin with electrophoretic mobility greater than -1.9×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}.
‡Although the descending electrophoretic pattern of top VIa showed no γ-globulin, the rising pattern indicated that the fraction contained about 2 per cent of this component. The patterns of top VIb and bottom VIb showed no γ-globulin, although the β-globulin peak did trail to a slight extent.

several fractions (Table II) were compared with their electrophoretic compositions. Reaginic activity was not appreciably associated with γ_2-globulin (γ-globulins with electrophoretic mobilities less than -1.9×10^{-5} cm.^{2} sec.$^{-1}$ volt $^{-1}$). Indeed, not only did fractions comprised largely of γ_2-globulin show negligible sensitizing activity, but other preparations containing no γ_2-globulin showed maximal activity. The same statement applies to the α-globulins and to albumin. For example, fraction BG exhibited low activity. This

TABLE II. NEUTRALIZATION REQUIREMENTS AND DILUTABILITY OF SEN 11/11/53 COTTONSEED-REAGINIC SERUM FRACTIONS
(Averages Obtained During Nine Passive Transfer Studies on Four Test Subjects)

FRACTIONS	ELECTROPHORETIC COMPOSITION (% β-GLOBULIN)	TOTAL PROTEIN (MG./ML.)	NEUTRALIZATION		DILUTABILITY	
			END POINT PNU/ML.	R_n* PNU/MG. TP	END POINT	R_d† EP/MG. TP
Whole serum	19	71.3	20.00	0.28	1:960	13.5
I	2	2.2	1.25	0.57	1:10	4.5
II	3	2.0	1.25	0.63	1:7.5	3.8
III	1	1.4	1.25	0.89	1:7.5	5.4
IV	9	1.3	2.50	1.93	1:15	11.5
Va	66	2.9	7.50	2.59	1:50	17.3
Vb	74	1.2	3.75	3.12	1:30	25.0
Vc	77	1.3	3.75	2.88	1:30	23.1
VIa	32	3.0	1.25	0.42	1:25	8.3
VIb	52	0.9	1.25	1.39	1:10	11.1
Bottom VIb	21	1.4	0.31	0.22	1:5	3.6
VII	21	3.4	1.25	0.37	1:15	4.4
BG	4	15.6	No transfer		1:1.3	0.1
BA	2	13.1	No transfer		No transfer	

*R_n = Neutralization ratio or units of antigen per 1 mg. total protein in the fraction.
†R_d = Dilution ratio or dilution end point per 1 mg. total protein in the fraction.

fraction contained small amounts of albumin and β-globulin, but was otherwise constituted entirely of α_1- and α_2-globulins. Similarly, fraction BA, which was chiefly albumin, was essentially inert.

The foregoing relationships eliminated each of the serum proteins except γ_1-globulin (γ-globulin of mobility -1.9×10^{-5} or greater) and β-globulin as major sources of reaginic activity. Since both of these components were present in three of the most active fractions, activity might have been associated with either constituent. However, when activity of the several fractions was plotted against their relative contents of γ_1-globulin and of β-globulin, it became apparent that reaginic activity was much more closely allied to β-globulin than the γ_1-globulin content. Indeed, one fraction that showed marked activity contained no detectable γ_1-globulin, but was composed solely of β-globulin, α_1-globulin, and albumin. Thus, β-globulin conferred sensitivity and did so in the absence of γ_1-globulin and α-globulins. Finally, the satisfactory general correlation noted between the activity and the β-globulin content of successive fractions justified the conclusion that reaginic activity was associated chiefly with these globulins (Figs. 2 and 3).

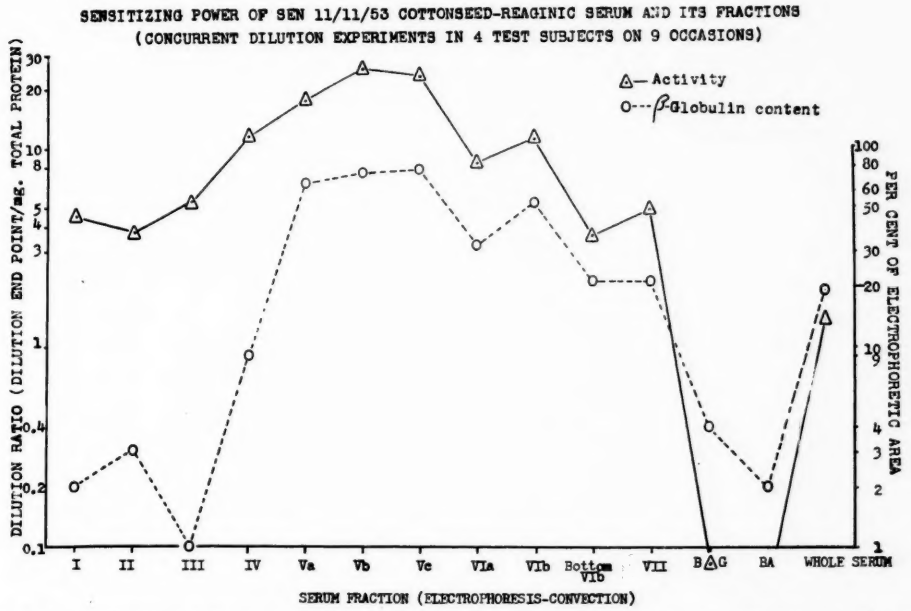

Fig. 2.—Correlation between sensitizing activity and β-globulin content. (Fractions of cottonseed-reaginic serum, SEN 11/11/53.) The former is represented by a *dilution* ratio.

The conclusion that the β-globulins of the SEN 11/11/53 serum were chiefly responsible for its reaginic activity was strongly supported by a supplementary experiment, in which top VIa was refractionated by electrophoresis-convection to yield two new fractions, top VIb and bottom VIb (Figs. 2 and 3). As shown in Fig. 4, the refractionation resulted in a redistribution of the proteins with a relative enrichment of top VIb and depletion of bottom

VIb with respect to β-globulins. Whereas the original top VIa contained 32 per cent β-globulins, top VIb acquired a final content of 52 per cent, while bottom VIb had only 21 per cent. The changes in reaginic activity paralleled these changes in β-globulin content to a striking degree. Thus, the reaginic activity ratios were 0.42 units of antigen per 1 mg. protein for top VIa, 1.4 for top VIb, and 0.22 for bottom VIb. These results can be seen in Figs. 2 and 3.

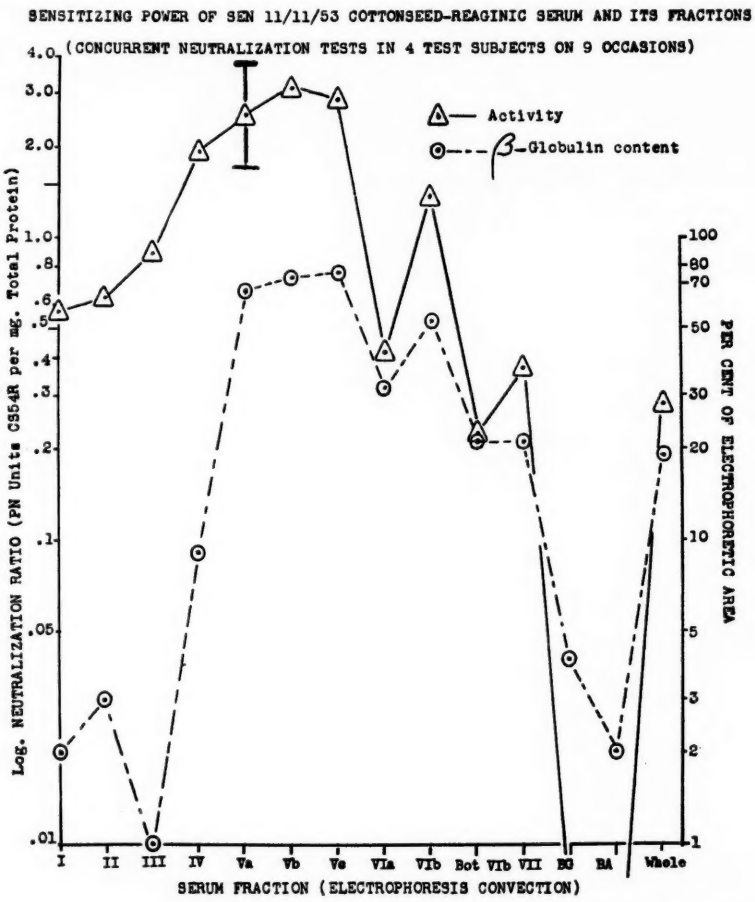

Fig. 3.—Correlation between sensitizing activity and β-globulin content. (Fractions of SEN 11/11/53 serum.) The former is represented by a *neutralization* ratio.

The foregoing findings for SEN serum are consistent with our published results[3-5] for four other reaginic sera. One of the latter possessed reagins for insulin, two for ragweed pollen, and the fourth was an earlier bleeding of SEN.

Blocking Serum.—The results of fractionation studies on blocking sera[5] stand in marked contrast to those obtained with reaginic sera. A representative case is that of patient B. E. R., an insulin-resistant woman. Her 2/3/53 serum was subjected to eight stages of electrophoresis-convection. Comparisons

made between the blocking activity and the electrophoretic composition of successive fractions revealed that blocking antibodies were not present in significant quantities in either the β-globulins or the albumin. Indeed, blocking activity was associated chiefly with the γ_2- and γ_1-globulins. Correlation studies showed that the only serum constituent to vary consistently with blocking activity was γ-globulin. Fig. 5 shows a positive correlation between the specific blocking power and the total γ-globulin content of the several fractions, in sharp contrast with the situation for β-globulin.

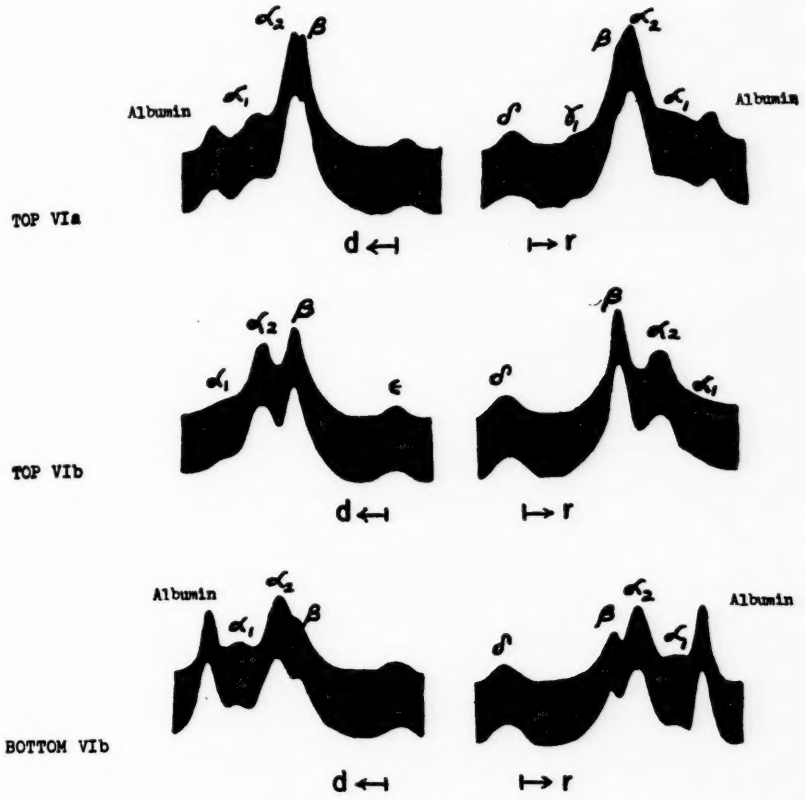

Fig. 4.—Refractionation of SEN top VIa to yield two new fractions, top VIb and bottom VIb.

Similar results were obtained with serum which contained both reaginic and blocking antibodies for ragweed pollen. Although the two activities overlapped in certain fractions, the reaginic activity followed the β-globulin content, whereas the blocking activity was correlated with the γ-globulin concentration.

DISCUSSION

Our conclusion that reaginic and blocking antibodies are distinct physical entities has been confirmed by the immunochemical investigations of Kuhns,[13, 14]

who used the diphtheria toxoid-antitoxin system. He found that human subjects who receive a single booster dose of diphtheria toxoid respond by forming one antibody or a mixture of two or three different antitoxins. The three varieties of antibody are (1) precipitating, (2) nonprecipitating and nonskin-sensitizing (blocking), and (3) nonprecipitating and skin-sensitizing (reagins).

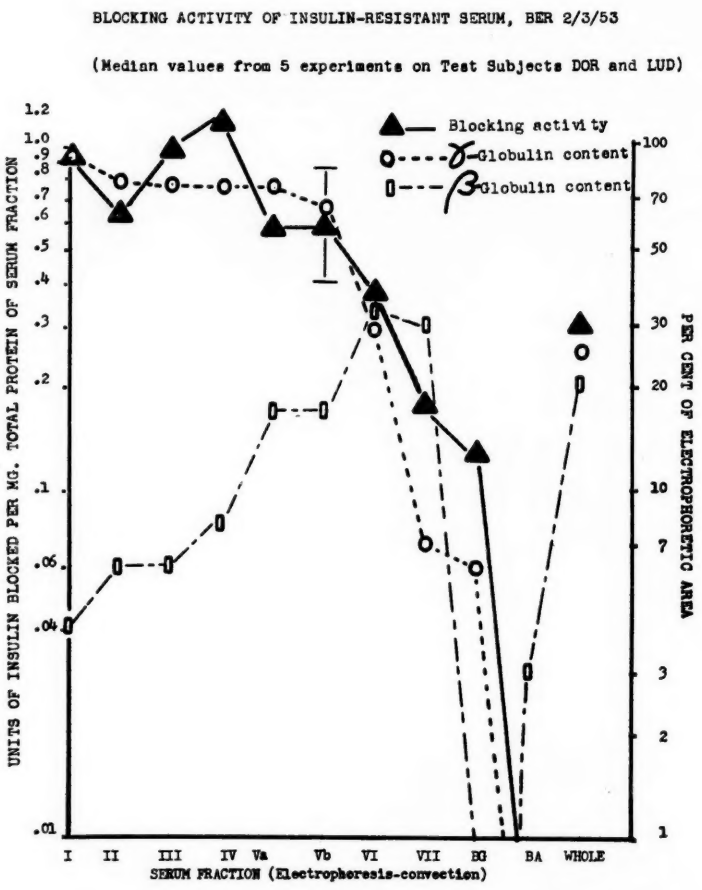

Fig. 5.—Correlation between *blocking activity* and electrophoretic composition. (Fractions of insulin-resistant serum, BER 2/3/53.) (From Loveless and Cann: *Journal of Immunology* 74: 329, 1955; reprinted with permission of the Williams & Wilkins Company.)

Kuhns has further shown that the reaginic antitoxin migrates electrophoretically as a γ_1-globulin, whereas both the precipitating and the blocking antitoxins migrate with the slower γ_2-globulin. The results reported by Berson and his co-workers[15] and by Peters and associates[16] also strengthen our conclusions regarding blocking antibody. Using I^{131}-labeled insulin, these workers showed that subjects injected with insulin for a number of months acquired in their serum a component which reacts specifically with insulin and which

migrates electrophoretically as a γ-globulin. It is our opinion that the acquired globulin is probably blocking antibody, comparable to the insulin-blocking factor noted in the γ-globulin fractions of our insulin-resistant sera.

Sehon, Fyles, and Rose[17] have reported recently on the electrophoretic properties of reagins in ten ragweed-allergic sera. The activity observed in fractions prepared by zone electrophoresis on a starch block would seem inconsistent with our results. Thus, they found that skin-sensitizing activity was associated with both the β- and the γ-globulin-enriched fractions, especially the latter. Unfortunately, their techniques do not lend themselves to quantitative interpretation. In four experiments, however, the original skin-sensitizing activity of the serum appeared to have been lost during fractionation. In a second series of fractionations, Sehon, Harter, and Rose[18] found ragweed reaginic activity associated with γ_1- and γ_2-globulins while fractions taken from the central portion of the β-globulin peaks were inactive. In some cases the distribution of sensitizing activity trailed into the β-globulins. As in their first series of experiments, the skin-sensitizing activity appeared to have been lost during the fractionation of two sera and was much diminished in a third sample. Thus, it seems likely that the starch-supporting medium used for their electrophoretic fractionations introduces technical complications which do not arise during fractionation by free electrophoresis. One explanation might be found in the fact that ragweed allergens contain carbohydrate.[19] This being the case, it is conceivable that the ragweed reagins studied by Sehon's group might have been bound to starch, thereby changing their electrophoretic mobility. Assuming that reagin is a β-globulin, this possibility could lead to one of several complications:

1. The reagins could be bound in a completely reversible manner to starch, but might migrate during zone electrophoresis at a slower rate than they ordinarily exhibit during free electrophoresis. In this case, the reagins would be eluted from the column along with the γ-globulins, rather than with the β-globulins. The former eluate might well exhibit reaginic activity, and its reagin might still migrate as a β-globulin during analysis with free electrophoresis. Under this circumstance, there would then be no conflict with our observations.

2. The reagin might be bound to starch in a reversible manner but, in so doing it might undergo structural changes which inflict a permanent change in electrophoretic mobility. In this case, active reagin might be eluted with the γ-globulins and might also migrate with them during free electrophoresis.

3. Reagin might be bound reversibly to starch but, as a consequence, lose its skin-sensitizing activity.

4. Reagin might be bound irreversibly to starch. It would then be absent from the eluates and the latter would be found inactive.

In fact, it is conceivable that several of these possibilities could be realized simultaneously. In this connection, it is interesting to note that C-reactive

protein migrates at a rate which is different during free electrophoresis than during starch electrophoresis.[20] Indeed, this protein, which appears in the serum during certain acute infections and which reacts with the C-polysaccharide of the pneumococcus, has the mobility of a β-globulin during free electrophoresis and of a fast γ-globulin during starch electrophoresis.

Finally, a word of caution is in order regarding the designation of specific electrophoretic components of serum as γ-, β-, and α-globulin. We might consider the globulin fraction of serum as possessing a continuous distribution of electrophoretic mobilities, the various maxima in the distribution being arbitrarily designated as γ_2-, γ_1-, β-, α_2-, and α_1-globulin. While these designations are useful, they should be employed with reservation. For example, the distribution of mobilities for γ_1- and for β-globulins probably overlaps in the region of their arbitrary division line. As a result, some protein molecules with mobilities intermediate between the mean mobilities of these two serum components could well be designated as either γ_1- or β-globulin, thereby causing confusion in the literature.

SUMMARY

Utilizing the method of electrophoresis-convection for the fractionation and refractionation of proteins in solution, we have shown by combined electrophoretic and immunologic study that reagins and blocking antibodies are distinct physical entities. Whereas sensitizing activity migrates electrophoretically with the β-globulins of serum, blocking activity moves with the γ-globulins.

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REFERENCES

1. Campbell, D. H., Cann, J. R., Friedman, T. B., and Brown, R. A.: Reagic Serum Fractions Obtained by the Electrophoresis-Convection Method, J. ALLERGY **21**: 519, 1950.
2. Campbell, D. H., Cann, J. R., Friedman, T. B., and Brown, R. A.: The Relation of Allergic Reagin to Electrophoretic Components of Serum, Science **119**: 289, 1954.
3. Loveless, M. H., and Cann, J. R.: Distribution of Allergic and "Blocking" Activity in Human Serum Proteins Fractionated by Electrophoresis-Convection, Science **117**: 105, 1953.
4. Cann, J. R., and Loveless, M. H.: Distribution of Sensitizing Antibody in Human Serum Proteins Fractionated by Electrophoresis-Convection, J. Immunol. **72**: 270, 1954.
5. Loveless, M. H., and Cann, J. R.: Distribution of "Blocking" Antibody in Human Serum Proteins Fractionated by Electrophoresis-Convection, J. Immunol. **74**: 329, 1955.
6. Kirkwood, J. G.: A Suggestion for a New Method of Fractionation of Proteins by Electrophoresis-Convection, J. Chem. Phys. **9**: 879, 1941.
7. Nielsen, L. E., and Kirkwood, J. G.: The Fractionation of Proteins by Electrophoresis-Convection, J. Am. Chem. Soc. **68**: 181, 1946.
8. Cann, J. R., and Kirkwood, J. G.: The Fractionation of Proteins by Electrophoresis-Convection, Cold Spring Harbor Symp. Quant. Biol. **14**: 9, 1950.
9. Cann, J. R., Brown, R. A., Kirkwood, J. G., Sturgeon, P., and Clarke, D. W.: Studies of Antibody Distribution in the Serum Proteins of Several Rh Antisera, J. Immunol. **68**: 243, 1952.
10. Timasheff, S. N., Moyer, A. W., Brown, R. A., and Kirkwood, J. G.: The Distribution of Polio Antibodies in Serum Proteins, Proc. Nat. Acad. Sc. **42**: 228, 1956.

11. Loveless, M. H., and Timasheff, S. N.: Fractionation of Ragweed Extract by Semicontinuous Electrophoresis-Convection, Arch. Biochem. & Biophys. **58:** 298, 1955.
12. Spies, J. R., Bernton, H. S., and Stevens, H.: The Chemistry of Allergens. IV. An Electrophoretic Fractionation of the Protein-Polysaccharide Fraction, CS-1A, From Cottonseed, J. Am. Chem. Soc. **63:** 2163, 1940.
13. Kuhns, W. J.: Immunochemical Studies of Antitoxin Produced in Normal Allergic Individuals Hyperimmunized With Diphtheria Toxoid. IV. Differences Between Human Precipitating and Non-precipitating Skin-Sensitizing Diphtheria Antitoxin as Shown by Electrophoresis, J. Exper. Med. **99:** 577, 1954.
14. Kuhns, W. J.: Immunological Properties of a Form of Non-precipitating Diphtheria Antitoxin Which Does Not Sensitize Human Skin; Studies of Its Relation to the Inhibition Reaction, J. Immunol. **75:** 105, 1955.
15. Berson, S. A., Yallow, R. S., Bauman, A., Rothschild, M. A., and Newerly, K.: Insulin-I^{131} Metabolism in Human Subjects: Demonstration of Insulin Binding Globulin in the Circulation of Insulin-Treated Subjects, J. Clin. Invest. **35:** 170, 1956.
16. Peters, T., Burrows, B. A., and Lowell, F. C.: Physical Binding of Insulin by γ-Globulins From Insulin-Resistant Subjects, Federation Proc. **15:** 608, 1956.
16a. Burrows, B. A., Peters, T., and Lowell, F.: Physical Binding of Insulin by Gamma Globulins of Insulin-Resistant Subjects, J. Clin. Invest. **36:** 393, 1957.
17. Sehon, A. H., Fyles, T. W., and Rose, B.: Electrophoretic Separation of Skin-Sensitizing Antibody From the Sera of Ragweed-Sensitive Patients, J. ALLERGY **26:** 329, 1955.
18. Sehon, A. H., Harter, J. G., and Rose, B.: The Localization of Skin-Sensitizing Antibody in the Sera of Ragweed Sensitive Individuals by Electrophoresis, J. Exper. Med. **103:** 679, 1956.
19. Loveless, M. H., Wright, I., and Ryan, A.: Allergenic Fractions of Low Ragweed Pollen. II. Some Immunologic, Electrophoretic, and Chemical Characteristics of Diffusates, J. ALLERGY **22:** 120, 1951.
20. Wood, H. F., McCarty, M., and Slater, R. J.: The Occurrence During Acute Infections of a Protein Not Normally Present in the Blood. V. Physical-Chemical Properties of the C-Reactive Protein Crystallized by a Modified Technique, J. Exper. Med. **100:** 71, 1954.

THE GENETIC BASIS OF BIOLOGICAL SPECIFICITY

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I PROPOSE to review very briefly some recent developments in our knowledge of the nature of hereditary material. This will be related, of course, to the subject of allergy, but in a rather remote way and certainly in a way that I am not prepared or qualified to discuss. So if you are expecting to learn anything of direct use in allergy, I am sorry I shall have to disappoint you.

We all know, of course, that genes make up the hereditary material. I can illustrate genetic characteristics in a very simple way with traits known in man. These also tell us something about what genes might do in a functional way.

HEREDITARY ANEMIAS

All of you know of the rather extensive recent work on the hemoglobins of man, starting with the so-called sickle cell hemoglobin that is differentiated from normal hemoglobin by a gene that is transmitted from one generation to another in the regular manner in which we know genes to be transmitted.

I can illustrate this by representing the normal form of the gene for hemoglobin with an open hand and the sickle cell counterpart of it (an allele) with a closed hand.

One has in each cell two representatives of this gene. They may both be normal, in which case a person has only normal hemoglobin. They may both be of the "S" type, in which case a person has only "S" hemoglobin. Or one may have one of each, the so-called heterozygous state, in which case there is a mixture of hemoglobins.

There are now known perhaps a dozen different hemoglobins. Hemoglobin "C," for example, represents another form of this same gene. We can represent the "C" form with a partly open hand.

With regard to the normal, "S" and "C" alleles, there are six possible genetic types. The hemoglobins present correspond exactly to the genes present in the cells, suggesting that the genes function more or less directly in determining the characteristics of the three types of hemoglobins.

Many examples could be given of this sort of relation. There has recently been reported what seem clearly to be hereditary differences in cattle as to

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whether they produce one or another form of beta-lactoglobulin. The two beta-lactoglobulins are detectably different chemically and appear to be inherited simply.

In the case of the sickle cell and "C" hemoglobins, it is now established that there are detectable differences in amino acid composition in the proteins of these two hemoglobins.

From these examples I should like to go to the question of what we know about the nature of the gene. To review this briefly, I shall remind you of a few elementary genetic principles.

NUCLEIC ACID NATURE OF GENES

Genes are carried in chromosomes. Chromosomes contain nucleic acid—deoxyribonucleic acid—and protein combined in a way that is not yet completely understood.

We know that mutations are produced by high energy radiation. Ultraviolet radiation produces mutations. Its absorption spectrum in the ultraviolet region is very closely correlated with the so-called mutation action spectrum; that is, the efficiency of ultraviolet in producing mutations per unit energy follows closely the absorption of ultraviolet by nucleic acid. This suggests that the energy responsible for mutations is absorbed by the nucleic acid of the chromosome, not by the protein.

Almost thirty years ago the transforming principles in pneumococci were first discovered. During the succeeding years, as you know, these agents in pneumococci by which specificity of polysaccharide capsule can be transferred from one type to another have been shown to be made up solely of deoxyribonucleic acid (DNA). The evidence for this is as good as the methods of modern physical chemistry and enzymology can make it.

VIRUSES

Even more spectacular evidence has come in recent years from the viruses. There are many viruses that have now been investigated. Perhaps the ones we know most about genetically are the bacterial viruses, or bacteriophages, that infect the ordinary colon bacillus.

The coliphages that have been most worked with have polyhedral heads about 800 A.U. long and tails about equal in length. Their life cycles go as follows:

Under appropriate conditions they are adsorbed to the surface of susceptible bacteria by the tips of their tails, presumably by an electrostatic pattern of amino and carboxyl groups on the tail of the virus and a mirror image pattern on the surface of the bacterium. The pattern is repeated many times on the surface of the bacterium, perhaps like a wallpaper pattern.

Through some process, possibly involving an enzymatic step, the bacterial virus injects its core into the host cell. The core is nucleic acid. The coat that stays outside is protein.

It is known that the core is injected and that the coat stays outside, within the limits of experimental error, by labeling the coat with sulfur^{35},

sulfur being contained in proteins but not in nucleic acids, or conversely, by labeling the DNA with phosphorus^{32}, which is found in nucleic acid but not in proteins.

When the label is in the protein the radioactivity stays outside. When it is in the nucleic acid it goes inside. About 97 per cent of the protein remains outside, possibly more. This is determined in a very simple way. After the process of infection is initiated, it is possible to knock the coats off in a Waring Blendor, separate them from the bacteria in a centrifuge, and then determine whether the radioactivity stays outside or goes inside. This now classical experiment was first done by Hershey and his collaborators at the Carnegie Institution of Washington laboratory at Cold Spring Harbor, New York.

The virus DNA then begins being replicated. At first two units of DNA are made, then four, and so on, until after ten or fifteen minutes there are perhaps 100 or 200 replicas of the DNA that was injected by the infecting virus.

Then the bacterial cell starts manufacturing proteins that are specific to the virus of the type belonging to the virus coat that did not go in.

Obviously the DNA must carry information into the bacterial cell that tells it what kind of protein to make. It normally would not make a virus protein, but under these conditions of infection it does.

Somehow, in a way not yet understood, the virus coats get put around the cores and after twenty minutes we get 200 or 300 descendant viruses like the original one. This is the most rapid known biologic reproduction—200 fold every twenty minutes. This would give an unbelievably large number of viruses in a few hours if one could save all the progeny and multiply them at this rate.

VIRUSES HAVE GENES

The viruses can be shown in a simple way to have hereditary units. For example, if one infects a large number of bacterial cells—a few million—with viruses to which they are susceptible, about one in a million of the bacterial cells will prove to be resistant, presumably as a result of an altered pattern of charges on the coat produced through mutation in the genetic material of the bacterial cell.

From such a resistant cell one can establish a bacterial line that is resistant to the particular virus used. If one then attempts to infect a very large number of these bacterial cells with an equally large number of viruses to which the cells are resistant, it is found that the virus, too, can undergo a mutational change in the pattern of electrostatic charges on its tail. Tail patterns that fit the new pattern on the bacterial cell replace the original parental cell type. This is mutation and natural selection in a very simple form.

It is also possible to find virus mutants that destroy bacteria at different rates from that of the normal strain. As a result, virus colonies on Petri plate culture heavily seeded with bacteria will be of various sizes or have other characteristics that are hereditary in the sense of being transmitted to successive generations.

With two such characteristics, for example, one that affects the pattern on the tail and one that affects the rate of destruction, it is possible to make crosses of two viruses that differ by these two hereditary traits. When one infects a bacterial cell simultaneously with two genetically different viruses, it is found that the progeny consists not only of descendants of the two that injected their DNA into the host cell, but two new types that represent recombinations of the traits of the parents. If there are two units of inheritance, there will be four types, the parental types plus the double mutant plus the original type that has no mutant at all.

This is exactly what happens in hybrids of higher organisms, and it is on this basis that geneticists define units of inheritance.

We can say, therefore, that bacterial viruses have units of inheritance in the same sense in which we have them. As a matter of fact, by doing this experiment one can measure the frequency with which recombination occurs, and this in turn is a function of the distance apart of the genes in the viral DNA.

By using the convention that 1 per cent of recombination equals 1 map unit, it is found that the total genetic material of a bacterial virus is 200 map units long. This value will be referred to later. It is about the length of one of the longer chromosomes of Drosophila.

Since it is clear from this type of study that bacterial viruses have genes and that DNA constitutes the only bridge between one generation of viruses and the next, it follows that DNA must constitute the primary genetic information. In other words, the gene must be reducible at one stage to DNA.

DEOXYRIBONUCLEIC ACID AS GENETIC MATERIAL

Let us now consider briefly what we know about DNA. To me this is one of the most interesting and significant chapters in modern biology.

Three years ago, as many of you know, Watson and Crick, then working at Cambridge, suggested a structure of deoxyribonucleic acid that is of particular interest from a biologic point of view because it accounts so nicely for the several most important properties of genetic material.

The Watson-Crick structure is based on the assumption that deoxyribonucleic acid in the natural state is made of two parallel polynucleotide chains running in opposite directions, hydrogen bonded together through their purine and pyrimidine bases, and coiled to form a regular helix.

There are two polynucleotide chains wound around a common axis. The chains consist of sugar-phosphate-sugar-phosphate. On each sugar is a purine or pyrimidine base directed inward. The four bases are designated as follows: A = adenine, T = thymine, G = guanine, and C = cytosine.

We can simplify the picture by imagining the helix unwound and stretched out essentially in two dimensions. A segment four base-pairs long is shown in Fig. 1. An essential property of the structure is that the two chains are

complementary in the sense that A is always paired with T and C with G. If one knows the sequence of bases in one of these chains, the other one can be read off as the complement.

GENETIC INFORMATION

DNA constitutes a kind of molecular code. Obviously one can spell out any kind of message in a sequence of base-pairs just as a message in Morse code can be represented as a sequence of dots and dashes. In DNA there are four symbols instead of two. Our alphabet enables us to code information in much the same way except that we use 26 symbols instead of four or two.

We believe that genetic information is spelled out in the sequence of these four base-pairs.

There are really only two base-pairs, but they can be turned over in the chain so there is an AT and TA pair. If you take one chain out, there are four single base-pairs, but this carries the same information as its complement just as a Morse code would carry the same information as a complementary Morse code if dash were substituted for dot and dot for dash. This is known as complementarity in coding.

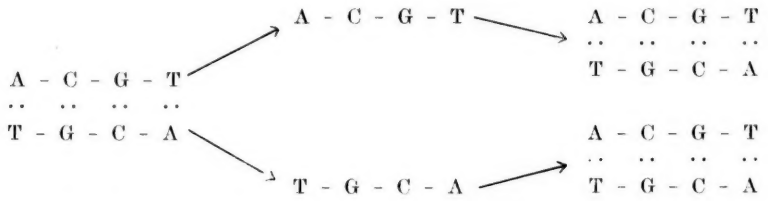

Fig. 1.—Schematic representation of duplication of a four-unit sequence of deoxyribonucleic acid. On the right are four base-pairs. Separation by rupture of hydrogen bonds (dots) is shown in the middle. On the right are two replicas of the original four-unit segment. Each half of the segment serves as a template for synthesis of its complement.

GENE REPLICATION

This complementarity gives a clue as to how genic material might be replicated, a question that biologists have wondered about from the beginning of genetics.

On working out their model Watson and Crick immediately made the suggestion that replication occurs by the separation of the two strands through the breaking of the weak hydrogen bonds by which they are held together through their common base-pairs.

Each single chain is believed to serve as a template in the building up of a complement. Thus, starting with a double structure, two complementary halves are formed. Each half directs the synthesis of a complement of itself with the result that after replication there are two double chains exactly like the starting double chain.

Supposedly this is the molecular basis of all biologic reproduction.

MUTATIONS—ERRORS IN REPLICATION

Genetic material is characterized by mutations. All living systems have evolutionary pasts and evolutionary futures which reside in the genetic material. The gene is believed to be the smallest unit capable of evolution. The Watson-Crick model explains how mutation might occur. If genetic information is represented by sequences of base-pairs, it follows that this information can be changed in ways analogous to the ways in which information in a written message can be changed. In copying a written message there is always a chance of an error of transcription—typographical errors in modern methods of machine writing.

When DNA is replicated it is theoretically possible to substitute one base-pair for another. In fact a mechanism has been suggested by Watson and Crick that will account for such substitutions.

The end result is as though you substituted in a written message one letter for another. This, of course, will often change the message in a very significant way—"now" for "not," for example.

Another possibility is that one or more base-pairs may be omitted during replication. This is analogous to leaving out a letter in a typewritten message.

Another kind of typographical error is duplication of symbols. A word or a letter may be written twice instead of once. There is genetic evidence that this type of error can be made on DNA replication. The result is known as duplication.

Still another possibility is that letters or segments may be transposed. Whole words may be transposed or one may be written backward. In either case the result is often a quite different message.

MUTATIONS LARGELY DELETERIOUS

Just as in a written message typographical errors often make nonsense, so in genetic material a mutation is likely to make nonsense. If a segment of DNA spells out a specific message, the probability is high that the message will no longer be useful if the sequence of symbols is scrambled.

But occasionally, just as a secretary can improve a letter by making a typographical error, a genetic message can be improved through a mistake in replication. The frequency with which the latter happens is very low, however. This is another way of saying that a favorable mutation is a very improbable event. It is most unlikely that a complexly organized system with a highly selected system of communicating information from generation to generation is going to be improved by accident. It is much less than the probability that dropping my watch will make it run better.

THE AMOUNT OF GENETIC INFORMATION

Let us now talk about how much of it there is. In a bacterial virus there are 20,000 turns of the double helix. Each turn has 10 base-pairs. There are therefore 200,000 base-pairs.

I pointed out previously that there are about 200 map units in the total genetic material of a bacterial virus; 200 units and 200,000 base-pairs mean 1,000 base-pairs per unit.

It is possible to identify a functional unit in the genetic material of a virus. This has been done now by several people by a technique that involves frequency of recombination within a gene. This is essentially like measuring recombination frequencies between genes.

It involves a hybrid with two defective genes of the same kind. If they are defective at different ends, it is possible to obtain good copies made up of the end of one gene and the other end of the second, thus reconstituting a functional gene from two defective ones.

The frequency of such intragenic recombination measures distance within genes just as ordinary genetic crossing-over measures distances between genes.

Professor Benzer of Purdue University has identified one locus that is presumedly concerned with the charge pattern on the tail of the virus. This unit is about 5 or 6 map units long. That probably means 5,000 or 6,000 base-pairs.

It is interesting to examine the question of how many ways 6,000 base-pairs can be arranged. If there were no restrictions in proportions, it is obvious that the total number of arrangements would be 4 raised to the six thousandth power. This is an inconceivably large number. It would be reduced greatly because many possible arrangements would have no conceivable biologic significance. It would be only the useful messages that would be saved by natural selection.

In each cell of our bodies there is perhaps 1,000 times as much DNA as in the head of a bacterial virus. And this then means that instead of 200,000 base-pairs, we have 200,000,000 base-pairs. This of course means that the total number of possible genetic types in an organism as complex as man is enormously greater than in a virus. It is not surprising, therefore, that no two of us are alike genetically.

The total information in a human cell nucleus makes up perhaps 10,000 to 100,000 genes, each gene being a segment of this DNA that spells out a message and is translated into action during development and functioning.

THE TRANSLATION OF GENETIC INFORMATION

I should now like to discuss the question of how the translation is made. How is the information in the DNA code transferred to other substances like proteins and polysaccharides?

Obviously there must be a mechanism by which the information in a segment of DNA is translated into that of a corresponding protein or other macromolecule.

It is presumed that a specific gene is responsible for the specificity of the globin of hemoglobin. If that gene is altered in a particular way, the amino acid sequence of the globin is modified in the manner characteristic of sickle

cell hemoglobin. Another type of modification is responsible for "C" hemoglobin and still others for the other known types of hemoglobin. An hypothesis that is currently held by many is that DNA information is somehow translated into information in the form of cytoplasmic ribonucleic acid (RNA), and that this in turn acts as a direct template for combining amino acids to form proteins.

This is one hypothesis. There is some experimental evidence to support it but it is by no means completely proved. In whatever way the translation is made, it is surely of the greatest importance to find out as much as possible about it.

In one sense, it can be said that the DNA in a fertilized egg of man constitutes a recipe for constructing a person out of a single cell plus some ten tons of food and a suitable environment. It is a complex recipe and we might well ask how many pages of printed directions it might correspond to. Crick has calculated this. His results can be expressed in the following way:

> We have 48 chromosomes. The DNA in one of these chromosomes is sufficient to encode a complete set of the *Encyclopaedia Britannica*. All 48 chromosomes are sufficient to encode about fifty times as much information as found in the *Encyclopaedia Britannica*. Stated in these terms the recipe for a man is the equivalent of about a thousand volumes as large as those of the *Encyclopaedia Britannica*.

In the past two or three years there have been several studies that attempt to resolve the so-called fine structure of the gene. It is possible that this might be done in terms of base-pair sequence just as in a few proteins such as insulin the amino acid sequence has been worked out. Although a number of investigators have attempted to "break" the DNA-protein code, no one has yet succeeded.

It is possible, however, to investigate the fine structure of the gene by means of intragenic recombination frequencies. By this method the smallest measurable interval between two mutational sites in a gene corresponds to about 12 A.U. of DNA or about four base-pairs. This suggests that the ultimate unit of mutation is what the hypothesis says it ought to be, a single base-pair.

From a functional standpoint some of us would like to think of the gene as a unit of DNA that corresponds to a specific macromolecule like a protein that is enzymatically active or that serves some other function.

On this basis it is not difficult to believe that for every macromolecule of the complexity of a protein there is a corresponding gene consisting of a segment of DNA. There is some experimental evidence of this hypothesis. For example, in the bacterium Salmonella, Demerec and his collaborators at the Carnegie Institution of Washington laboratory at Cold Spring Harbor, New York, have made a large series of mutants that are defective in specific ways. They are unable to carry out specific biochemical reactions such as the synthesis of the amino acid tryptophane. Four steps in the synthesis of tryptophane have been identified. A large number of salmonella strains unable to make

tryptophane have been classified as to which of the four steps is blocked. All of those blocked in the final step are found to depend on changes in a single gene. Those that are blocked in the penultimate step likewise depend on changes in a single gene but a different one from that concerned with the final step. The same relation holds for the first two steps.

There is now an impressive array of evidence consistent with the prediction that mutants concerned with the activity of a particular enzymatic protein should be differentiated from normal by changes in a single gene. There are a few exceptions that we do not yet understand. It cannot yet be said whether or not they are of real significance.

SUMMARY

I shall conclude with the summary statement that it is now a widely accepted working hypothesis that the primary genetic material of higher organisms is deoxyribonucleic acid, and that the structure proposed by Watson and Crick for this substance seems to provide a sensible chemical basis for the specificity, replication, mutation, and functioning of genetic material.

REFERENCES*

Beadle, G. W.: The Role of the Nucleus in Heredity, *In* A Symposium on the Chemical Basis of Heredity, edited by W. D. McElroy and B. Glass, Baltimore, 1957, The Johns Hopkins Press, pp. 3-22.

Crick, F. H. C.: The Structure of the Hereditary Material, Scient. Am. **191:** 54-60, 1954.

Demerec, M., Hartman, Z., Hartman, P. E., Yura, T., Gots, J. S., Ozeki, H., and Glover, S. W.: Genetic Studies With Bacteria, Washington, 1956, Carnegie Institution of Washington, Publ. 612.

Ephrussi-Taylor, H.: Genetic Aspects of Transformations of Pneumococci, Cold Spring Harbor Symp. Quant. Biol. **16:** 445-455, 1951.

Haldane, J. B. S.: The Biochemistry of Genetics, New York, 1954, The Macmillan Co.

Hershey, A. D.: Inheritance in Bacteriophage, Advances in Genetics **5:** 89-106, 1953.

Horowitz, N. H.: On the Evolution of Biochemical Syntheses, Proc. Nat. Acad. Sc. **31:** 153-157, 1945.

Ingram, V. M.: A Specific Chemical Difference Between the Globins of Normal Human and Sickle-Cell Anaemia Haemoglobin, Nature **178:** 792-794, 1956.

Lederberg, J.: Genetic Transduction, Am. Scientist. **44:** 264-280, 1956.

Luria, S. E.: Bacteriophage: An Essay on Virus Reproduction, Science **111:** 507-511, 1950.

Luria, S. E.: General Virology, New York, 1953, John Wiley & Sons.

Lwoff, Andre: The Life Cycle of a Virus, Scient. Am. **190:** 34-37, 1954.

Muller, H. J.: The Gene, Proc. Roy. Soc., London, s.B. **134:** 1-37, 1947.

Neel, J. V., and Schull, W. J.: Human Heredity, Chicago, 1954, University of Chicago Press.

Srb, A. M., and Owen, R. D.: General Genetics, San Francisco, 1952, W. H. Freeman & Co.

Stern, Curt: Principles of Human Genetics, San Francisco, 1949, W. H. Freeman & Co.

Thorup, O. A., Itano, H. A., Wheby, M., and Leavell, B. S.: Hemoglobin, J. Science **123:** 889-890, 1956.

Wagner, R. P., and Mitchell, H. K.: Genetics and Metabolism, New York, 1955, John Wiley & Sons.

Watson, J. D., and Crick, F. H. C.: The Structure of DNA, Cold Spring Harbor Symp. Quant. Biol. **18:** 123-131, 1953.

Wright, S.: The Physiology of the Gene, Physiol. Rev. **21:** 487-527, 1941.

*No attempt is made to give an exhaustive list of references. Those listed here are chosen because of their general nature or because they report recent developments of importance.

DIFFERENTIATION OF CHRONIC GALACTOSEMIA AND MILK ALLERGY IN EARLY CHILDHOOD

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AMONG causes for vomiting, colic, and diarrhea in the newborn infant, food allergy is common, especially that due to cow's milk. The relief of the child's difficulties by the elimination of milk need not necessarily lead to the diagnosis of milk allergy, however.

This paper aims to call attention to a disease which has recently become quite well known in pediatric circles. In 1908 von Reuss[7] and in 1917 Goeppert[1] observed galactosemia and galactosuria in certain children after the ingestion of milk and other foods containing galactose. This disease seems to be of hereditary origin, the result of a single recessive gene or of a more complex genetic pattern.[4] The specific enzymatic defect in galactosemia is the absence of an enzyme, phosphate-galactose-uridyl-transferase, which is responsible for the transfer of galactose-1-phosphate to glucose-1-phosphate.[5, 6]

These children frequently present themselves with gastrointestinal disorders such as diarrhea, vomiting, or abdominal distention.[2] Malnutrition, hepatomegaly, jaundice, splenomegaly, lamellar cataracts, mental retardation, and proteinuria are all occasional features of this disease called galactosemia or galactose diabetes.[3]

The diagnosis is frequently missed, because such a child in the hospital is given only glucose water, parenteral fluids, or nothing by mouth, leading therefore to the absence of reducing substances in the urine.

Some of the defects mentioned occur only if the disease has been allowed to progress undetected for any length of time. The usual disappearance of all subjective and objective symptoms on a milk-free diet, with substitution by soybean, meat-base, or protein formulas, is dramatic and leads to the need for pointing out differential diagnostic points between this disease and milk allergy, as described in Table I.

The diagnosis of chronic galactosemia is proved by the presence of abnormal amounts of galactose in the urine and blood, present only if the child has ingested foods containing galactose. Galactose is not fermented by yeast in contradiction to glucose and thus can be differentiated from the latter. The addition of phenylhydrazine to galactose yields typical galactosazone crystals.

Many of these points are illustrated by the following case from U. S. Army Hospital, Fort Knox, Kentucky.

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TABLE I. DIFFERENTIAL DIAGNOSTIC POINTS

SYMPTOMS	CHRONIC GALACTOSEMIA	MILK FOOD G.I. ALLERGY
Nausea	May be present	May be present
Vomiting	May be present	May be present
Colic	May be present	May be present
Diarrhea	May be present	May be present
Failure to gain weight	May be present	May be present
Jaundice	Usually present	Absent
Hepatomegaly	Usually present if disease of longer duration	Absent
Lamellar cataracts	Usually present if disease of longer duration	Absent
Mental retardation	Usually present if disease of longer duration	Absent
Proteinuria	Usually present	Absent
Galactosuria	Present if child on milk	Absent
Symptoms present if child is fed	Any milk, including human milk	Only one species-specific milk
Symptoms disappear on cessation of milk feedings	Yes	Yes
Galactose tolerance test	Elevated, abnormal	Normal
Skin test to cow's milk	Negative	Sometimes positive
Family history of allergy	Usually no	Usually yes
Family history of galactosemia	Sometimes yes	No

H. A. W. was a full-term white male infant, born on July 31, 1956, to a primiparous mother. The past history of the parents, the prenatal course, and the delivery were noncontributory except that the mother's catherized urine specimen taken at the delivery table contained a trace of sugar. There was no family history of allergies or of symptoms which could have been the result of chronic galactosemia.

The physical examination of the infant one day after delivery failed to reveal any physical abnormalities.

The child was breast-fed by the mother and received no supplemental cow's milk feedings. There was no fever, diarrhea, or excessive crying. On August 3, the child appeared jaundiced, but this was judged to be due to physiologic jaundice and the child was discharged on the fourth day. Laboratory work-up showed no evidence of Rh, ABO, MN, or any other blood incompatibility; both the child and the mother were O, Rh positive, and the direct Coombs' test was negative. On August 10, the child was readmitted to the hospital because of diarrhea for three days and inability to take an evaporated milk formula (breast-feeding was discontinued after discharge from the hospital), and jaundice of six days' duration. Physical examination at this time showed a weight of 6 pounds, 1½ ounces, compared with a birth weight of 7 pounds, 2½ ounces. The child appeared jaundiced and malnourished, but liver and spleen were not enlarged; the lenses appeared clear and an ophthalmologist failed to detect any lamellar cataracts. The diagnosis on admission was acute gastroenteritis.

The initial laboratory results were: *urine:* trace of albumin, sugar 3 plus, being a nonglucose reducing sugar, 13 to 16 W.B.C. and 4 to 5 R.B.C.; *blood:* R.B.C. 5, 45 W.B.C. 14,550, neutrophils 64, leukocytes 36, hemoglobin 18.6, with no nucleated red cells on the peripheral smear. The total bilirubin on admission was 27.4 mg. per cent, with 20.9 mg. per cent being indirect and 6.5 mg. per cent being direct; the stools appeared yellow and bile-stained.

The child was placed on a mixture of equal parts of 10 per cent Arobon solution and Lytren feedings,* which led to the control of the diarrhea in two days. The child appeared "quiet between feedings" on the third day of treatment and also appeared less jaundiced.

*Arobon is carob powder produced commercially by Nestlé products, White Plains, New York; Lytren is an electrolyte solution produced commercially by Mead Johnson & Co., Evansville, Indiana.

Urinalyses on August 13 and 14 were negative for sugar, and on August 14, the child was placed on Nutramigen* feedings ad lib. He started to gain some weight and there was no jaundice present on August 16. Evaporated cow's milk formula was started on August 16 and within three hours galactosuria was noted and was present in each urine specimen while the child was given milk.

On this diagnostic trial the diarrhea and vomiting recurred and the child stopped gaining weight. Frequent crying and colicky behavior were noted. After seven days on milk there was, however, no recurrence of the jaundice. The trial was stopped and the urine became negative for sugar. The child was first placed on equal parts of 10 per cent Arobon-Nutramigen, and, after cessation of the diarrhea, on plain Nutramigen. Two galactose tolerance tests were performed. On one occasion 1.75 Gm. of galactose per kilogram of body weight was administered by bottle in form of a galactose-water solution after the bladder was emptied. A four-hour total urinary output yielded 1.24 Gm. of galactose (normal for newborn infants, less than 1 Gm). On another occasion the same procedure was employed. The results are seen in Table II.

TABLE II

	BLOOD GALACTOSE IN MG. PER CENT	URINE "SUGAR"
Fasting	0	Negative
½ hour	142	+++
1 hour	76	+++
2 hours	151	+++

The blood galactose levels were obtained by first determining total blood sugar levels and then subjecting this blood sugar to yeast fermentation. The undigested component was interpreted to be galactose. Multiple blood and urinary specimens were also examined with phenylhydrazine and yielded typical galactosazone crystals.

On discharge August 31, he was placed on a milk-free diet and has done well other than for a minor U.R.I. and diarrheal episode in October. He is alert and cooperative; no mental defect is apparent, the eyes show no evidence of cataracts, and periodic urinalyses reveal no sugar. At no time was there hepatomegaly or splenomegaly. A recent emergency operation for an incarcerated inguinal hernia was performed. His only problem at present is difficulty in gaining weight and a slight hypochromic microcytic anemia.

DISCUSSION

Since early diagnosis of galactosemia can prevent irreparable damage such as mental retardation, cataracts, and hepatomegaly, practicing physicians should be on the alert for such a possibility. The initial symptoms may mimic milk allergies in infants, and it is wise to do urinalyses in every case of suspected milk allergy and galactosemia. Since children admitted for diarrhea or gastroenteritis are usually placed on milk-free electrolyte feedings, a negative urine analysis may be misleading. Galactosuria occurs only when the child has ingested galactose; therefore, the diagnosis will be easily missed on a milk-free diet. Just as a diagnostic trial on milk is used to prove milk allergy, it is used likewise to prove galactosemia, and the importance of urinalysis and galactose tolerance tests should be equally important as specific allergy test procedures.

*Nutramigen is a commercially prepared hypoallergenic formula, containing a pancreatic hydrolysate of casein combined with carbohydrate, fat, and minerals but free of lactose or galactose. (Manufactured by Mead Johnson & Co., Evansville, Indiana.)

Jaundice aids in the diagnosis of galactosemia and its occurrence in the presence in a milk allergy would require a work-up involving studies for galactosemia as well as liver disease. A child with milk allergy, failure to gain weight, and appearance of mental retardation or cataracts should likewise be studied for galactosemia.

Helpful features in the diagnosis of galactosemia are the usual absence of a family history of allergies and sometimes a history of similar members of the family being blind or jaundiced in early childhood, pointing to familial galactosemia. Galactosemia occurs in children who are fed any milk, including human milk, which is obviously not the case in milk allergies to a species-specific milk antigen.

SUMMARY

Gastrointestinal milk allergies in early childhood should be differentiated from chronic galactosemia, which is characterized by galactosuria and usually by vomiting, colic, diarrhea, failure to gain weight, jaundice, hepatomegaly, lamellar cataracts, and mental retardation. One case has been presented. The early diagnosis of chronic galactosemia may prevent most of the serious complications.

REFERENCES

1. Goeppert, F.: Galactosurie nach Milchzuckergabe bei angeborenem, familiarem, chronischem Leberleiden, Berliner Klin. Wchnschr. **54**: 473, 1917.
2. Bain, H. W., et al.: Galactosemia, A. M. A. Am. J. Dis. Child. **88**: 651, 1954.
3. Nelson, W. E.: In Mitchell-Nelson Textbook of Pediatrics, Philadelphia, 1950, W. B. Saunders Co., p. 293.
4. Holzel, H., and Komrower, G. M.: Study of the Genetics of Galactosaemia, Arch. Dis. Child. **30**: 155, 1955.
5. Isselbacher, K. J., et al.: Congenital Galactosemia, a Single Enzymatic Block in Galactose Metabolism, Science **123**: 635, 1956.
6. Isselbacher, K. J., et al.: The Specific Enzymatic Defect in Galactosemia, A. M. A. J. Dis. Child. **92**: 458, 1956.
7. Von Reuss, A.: Zuckerausscheidung im Säuglingsalter, Wien. med. Wchnschr. **58**: 799, 1908.

THE EFFECT OF CORTICOSTEROID ADMINISTRATION UPON BLOOD HISTAMINE CONTENT

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THE striking effect of corticosteroids upon clinical manifestations of hypersensitivity has suggested to various investigators that a study of histamine metabolism during administration of corticosteroids might add significantly to knowledge of hypersensitivity, as well as to knowledge of the working mechanism of corticosteroids used therapeutically in allergic disease.

The observations reported here concern the effect of corticosteroids upon the histamine content of whole blood. Since the blood histamine level has been thought in the past to vary largely, if not entirely, with the leukocyte count,[1] histamine level fluctuations are compared with changes in total leukocyte counts and in constituent cell types to determine whether changes in these values are comparable.

METHOD

Patients selected were started on corticosteroid therapy, either for the first time or after a considerable lapse of time since previous corticosteroid therapy. With two exceptions, the patients were asthmatic.

A control venous blood sample was taken a few hours prior to the start of therapy, and additional samples were obtained at varying intervals thereafter until it seemed that some steroid effect, as indicated by the patient's clinical status, might have been achieved. A small amount of heparin solution was used as an anticoagulant.

Total and differential leukocyte counts were done on the blood samples. Total counts were made with Thoma pipettes, using a 1:10 dilution. Cells on four large squares of a Neubauer hemocytometer were counted. Differential counts were done on thin slide films, using Wright's stain. Slides were scanned with the 4 mm. objective for distribution of cells. Differential counts for Patients 1 to 8 were obtained by identifying 100 cells and for Patients 9 to 11 by identifying 1,000 cells. Whole blood histamine was determined by the method of Lowry and Graham.[2] In some instances plasma histamine values were also obtained.

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Dr. Helen Graham's advice has been most helpful in interpreting the results of these studies.

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TABLE I. BLOOD HISTAMINE VALUES DURING CORTICOSTEROID ADMINISTRATION

PATIENT	DAY	TOTAL W.B.C.	DIFFERENTIAL W.B.C.						PLASMA HISTAMINE (MCG./L.)	WHOLE BLOOD HISTAMINE (MCG./L.)	STEROID	TOTAL DOSE OF STEROID (MG.)	TOTAL WHITE CELLS (% CHANGE)	WHOLE BLOOD HISTAMINE (% CHANGE)
			EOSINOPHILS	BASOPHILS	STAB.	SEGS.	LYMPHOCYTES	MONONUCLEARS						
1	−2	7,500	2	–	5	66	20	7	3.5	37				
Arthritis	1	8,100	–	–	–	–	–	–	–	44				
	2	9,300	–	–	5	60	29	6	5	35	Prednisone	25	+15	−20
2	−7	9,100	7	–	4	49	38	2	6	106				
Asthma	1	7,900	2	–	5	59	30	4	2	105				
	6	12,600	–	1	5	53	37	4	3	140	Prednisone	105	+59	+33
3	1	12,200	7	1	6	51	29	6	5	70				
Asthma	3	9,500	10	–	3	55	30	2	3	58	Prednisone	60	−22	−17
	4	12,700	1	–	4	55	34	6	2	60		80	+ 4	−14
	10	7,500	1	–	4	56	35	4	3	93		130	−38	+33
4	–	13,700	36	–	2	30	31	1	3	180	20 units			
Eosinophilia,	2	12,400	18	–	5	42	31	4	5	164	ACTH I.V.		− 9	−10
cause	4	11,000	13	2	3	48	30	4	3	155	on first day		−20	−14
undetermined														
5	0	10,100	1	–	7	50	38	4	7	71	Prednisone			
Asthma	3	10,000	1	–	7	63	25	4	2	63		90	− 1	−11
	7	13,300	1	–	4	63	30	2	5	75		180	+32	+ 6
	10	12,500	1	–	6	56	29	8	4	63		240	+24	−11
6	1	7,700	7	–	3	56	32	2		65	Prednisone			
Asthma	3 hours	13,000	–	1	7	73	18	1		65		30	+69	0
	3	10,600	1	–	5	73	19	2		47		40	+37	−28
	6	8,600	4	–	2	71	20	3		52		55	+12	−20
	14	10,300	4	–	3	74	18	1		57		85	+34	−12
7	1	8,600	10	2	2	43	38	5		72	Prednisone			
Asthma	2	9,200	2	–	2	61	32	3		39		30	+ 7	−46
	3	6,500	2	–	5	54	32	7		43		35	−24	−40
	7	5,100	9	1	2	50	32	6		42		55	−40	−41
8	1	5,700	3	–	1	52	42	2		35	Prednisone			
Asthma	3 hours	6,400	1	–	5	75	17	2		35		30	+12	0
	2	8,600	1	–	1	68	28	2		20		35	+51	−43
	8	7,900	2	1	3	58	30	6		46		65	+39	+31

RESULTS

Results of investigations in eight patients are reported in Table I.

Patient 1 had severely deforming chronic rheumatoid arthritis. Twenty-four hours after prednisone therapy was started, there was a slight increase in total leukocytes with a decrease in whole blood histamine of 20 per cent, as compared to the control level obtained on the day therapy was initiated. Evidently this change represents a mild response to a relatively small dose of prednisone.

The second patient, an asthmatic who was treated by other measures for one week before prednisone was started, enjoyed complete remission during the first week of steroid therapy. Histamine content of the first two blood samples, obtained before starting prednisone, was almost identical. After treatment, the blood histamine content actually increased, but not at the same pace with the total white blood cell count. This increase in blood histamine occurred in spite of a drop in eosinophils from 7 to 0 per cent.

The third patient, an asthmatic, showed a similar marked drop in blood eosinophilia on the fourth day of treatment, but only a slight diminution in the blood histamine concurrently. As in the previous patient, there was an increase in the histamine value of the final blood sample obtained.

In the case of the fourth patient,* observations during hospitalization and for a period of six months since have not revealed the cause of the marked eosinophilia. White cell differential determinations showed a 55 per cent decrease in eosinophils in the first twenty-four hours following intravenously administered corticotrophin. This decrease may be calculated in absolute numbers as a drop from 4,932 to 2,232 eosinophils per cubic millimeter. It was accompanied by a drop of 10 per cent in the blood histamine content. After an additional twenty-four hours, examination of the blood showed 1,430 eosinophils per cubic millimeter, and concomitantly there was an additional slight decrease in blood histamine content.

The fifth patient, who had asthma and urticaria, showed a slight over-all drop in blood histamine levels, in spite of a moderate increase in total white blood cell count.

In all five of these patients, plasma histamine values were determined, and in no instance did any remarkable change in plasma histamine values occur with steroid administration.

In Patients 1, 2, 3, and 5, who were given prednisone, individual doses of 5 or 10 mg. were used, to a total daily dose of 15 or 20 mg. Patients 6 and 7, both asthmatic, were treated differently in that an initial dose of 30 mg. of prednisone was given, followed by a daily dose of 5 or 10 mg. during the period when observations were in progress. This change in dosage schedule seemed to produce a more marked change in the blood picture, as well as prompt therapeutic response.

In the sixth patient 30 mg. of prednisone was administered immediately after the control blood sample was obtained. Three hours later the second

*Dr. S. C. Bukantz kindly permitted the studies on this patient.

blood sample showed a marked neutrophilic leukocytosis, with disappearance of the moderate eosinophilia, and no change in the whole blood histamine value.

Initially there were few eosinophils in the blood of the eighth patient. Three hours after administration of 30 mg. of prednisone, a decrease in their numbers did occur, without change in the whole blood histamine value.

In the seventh patient, a three-hour blood sample was not obtained. A drop in the number of eosinophils was evident on the day following the initial dose of prednisone, accompanied at this time by a considerable depression of the blood histamine content. On the seventh day of treatment the degree of eosinophilia approached the control value, although there was very little recovery toward the control blood histamine value.

Blood histamine and blood cell observations were carried out in Patients 9, 10, and 11 for a twenty-four-hour period. A control blood specimen was obtained, and immediately thereafter 30 mg. of prednisone was given by mouth. At three-, four-, and twenty-four-hour intervals after prednisone, additional blood samples were obtained. Results in these three patients are shown in Table II, in which the total numbers of each white cell type, calculated from the 1,000-cell differential count and the total count, have been included. Beneath the figures for total numbers of each cell type, theoretic values for the contributions of each cell type to the total blood histamine are shown in parentheses. These values are derived by using the average normal values established by Graham for basophils (1,080 mcg. histamine per 10^9 cells), eosinophils (160 mcg. per 10^9 cells), neutrophils (3 mcg. per 10^9 cells), and mononuclears (0.6 mcg. per 10^9 cells).[3]

The disappearance of large numbers of eosinophils from the blood within three hours after prednisone administration is apparent. In the same time period, the numbers of basophils were not significantly altered, however. At the end of four hours the basophils had significantly diminished and eosinophils had continued their decline. In twenty-four hours both eosinophils and basophils were present in numbers approximating pretreatment values.

DISCUSSION

Much attention has been given to the behavior of leukocytes following administration of corticotrophin or adrenal steroids. In general, it has been established that the typical early response is a neutrophila, at times an actual neutrophilic leukocytosis, with a concurrent diminution in the numbers of eosinophils and basophils.[4-6]

One mechanism which may explain the increase in numbers of neutrophils is the inhibition of leukocytic diapedesis, which is a known corticosteroid effect.[7] Why such an increase does not occur in the case of eosinophils, which are also diapedetic, is not known.

In Patients 6, 8, 9, 10, and 11 observations confirm that neutrophilia follows within three hours the administration of doses of readily absorbed corticosteroid which are usually therapeutically effective against allergic reactions.

In Patient 6 (see Fig. 1 for a graphic comparison), leukocytosis with a neutrophilia of 80 per cent was present three hours after the initial dose of

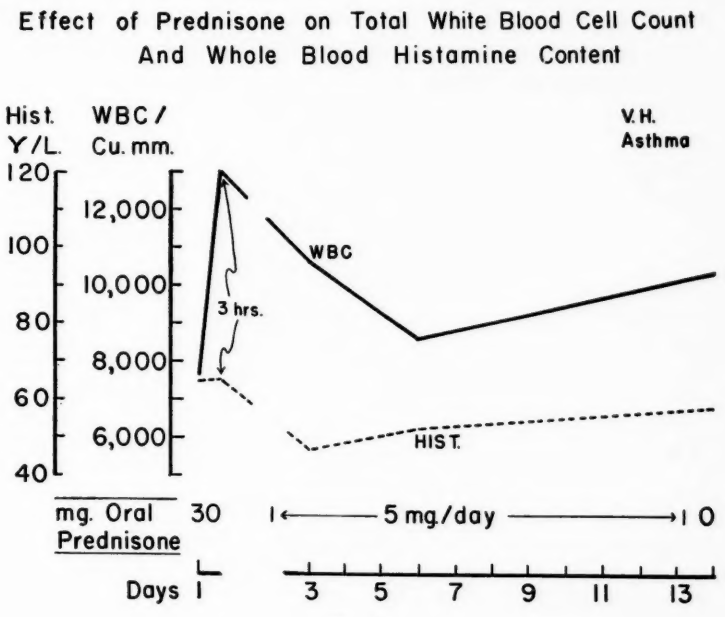

Fig. 1.

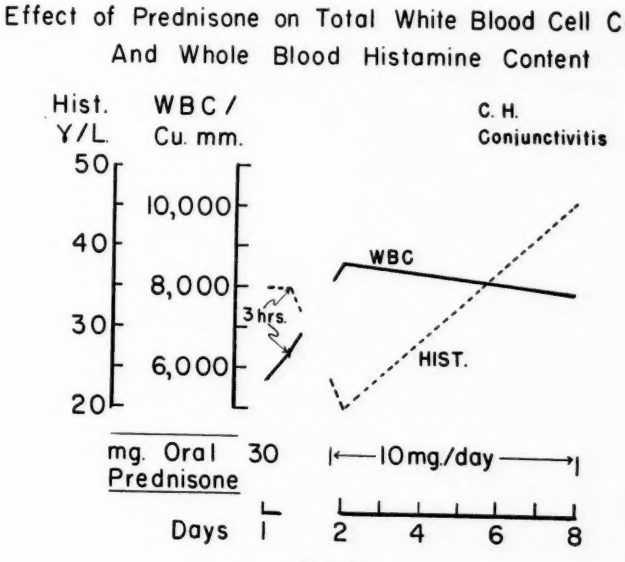

Fig. 2.

TABLE II. WHITE BLOOD CELL CHANGES AND BLOOD HISTAMINE VALUES, ACTUAL AND CALCULATED, AFTER 30 MG. PREDNISONE ORALLY

PATIENT NO.	9				10				11			
Time (hours) after prednisone	0	3	4	24	0	3	4	24	0	3	4	24
Total W.B.C./c.mm.	5,300	11,500	10,500	7,200	5,500	10,700	6,600	6,200	11,300	14,900	13,500	10,500
Actual total histamine (mcg./L.)	75	63	59	94	133	122	73	106	69	68	55	76
Total basophils/cu.mm.	21	34	11	43	50	54	26	68	79	75	54	74
(calculated histamine mcg./L.)	(22.7)	(36.8)	(11.9)	(46.4)	(54)	(58.3)	(28.1)	(73.4)	(85.3)	(81)	(58.3)	(79.9)
Total eosinophils/cu.mm.	159	58	11	158	517	161	20	459	893	268	176	777
(calculated histamine mcg./L.)	(25.4)	(9.3)	(1.8)	(25.3)	(82.7)	(25.6)	(3.2)	(73.6)	(142.9)	(42.9)	(28.2)	(124.3)
Total neutrophils/cu.mm.	3,313	9.971	9,218	3,945	3,514	9,587	6,131	3,782	6,486	12,695	11,313	5,691
(calculated histamine mcg./L.)	(9.9)	(30)	(27.7)	(11.8)	(10.5)	(28.8)	(18.4)	(11.3)	(20.5)	(38.1)	(33.9)	(17.1)
Total mononuclears/cu.mm.	1,807	1,437	1,260	3,055	1,419	898	422	1,891	3,842	1,862	1,958	3,959
(calculated histamine mcg./L.)	(1.1)	(1)	(1)	(2)	(1)	(1)	(0.2)	(1)	(2)	(1)	(1)	(2)
Total calculated histamine (mcg./L.)	59	77	42	86	148	114	50	159	251	163	121	223

prednisone. In spite of a rise of over 100 per cent in the absolute number of neutrophils, the blood histamine content was unchanged. With 5 mg. of prednisone daily, blood histamine remained at a level lower than the original.

In Patient 8 (Fig. 2), neutrophils increased from 53 to 80 per cent in the first three hours without change in the whole blood histamine. Twenty- four hours after prednisone was started, the patient's blood contained almost twice as many neutrophils as originally, but with a decrease of 43 per cent in the original blood histamine value.

The finding that neutrophilia is not accompanied by elevation of the blood histamine level, originally noted by Code,[1] is in agreement with the conclusion of Graham and associates that neutrophils contain an insignificant amount of histamine, calculated to amount to an average value of only 3 meg. of histamine in 10^9 neutrophils.[7] By contrast, 160 meg. per 10^9 cells was found to be the eosinophil histamine content, and 1,080 meg. the histamine present in 10^9 basophils. Graham states, however, that "it now seems possible that not only eosinophils but all types of granulocytes may contain quite different concentrations of histamine under different conditions." This is in agreement with the earlier observations of Code, and also with our observations, for in those cases in Table I in which a significant change in numbers of eosinophils permits a rough estimate of the histamine content of these cells, the values obtained are much lower than 160 meg. per 10^9 cells, varying from 50 meg. to none per 10^9 cells.

However, since in these instances we have not made accurate determinations of the basophils present and since it is known, as stated previously, that the basophil contains much larger amounts of histamine than any other leukocyte, it may be that the changing numbers of basophils present have caused these apparent variations in eosinophil histamine.

In considering such a possibility, the observations of Code and his associates[6] are helpful. Their findings indicate that cortisone reduces the number of basophils in the blood of normal human beings in a manner similar to that in which it reduces the number of eosinophils. In observations done at twelve- or twenty-four-hour intervals, they were able to determine that histamine levels always diminished with diminution of eosinophils.[8] However, some of our findings, previously noted, indicate that a considerable reduction may take place in the number of eosinophils without significantly altering blood histamine values.

While the basophils respond to corticosteroids with a decrease in numbers, as do the eosinophils, our data indicate that the response may be somewhat more sluggish than that shown by eosinophils. In this connection, Mitchell's[9] observations on a patient given corticosteroids are interesting. In studying the transport of injected histamine in a patient's blood, Mitchell had occasion to do hourly basophil and eosinophil counts after administration of cortisone. The decrease in eosinophils started within two hours after administration of cortisone by mouth and was precipitous, from about 250 to a few cells per cubic millimeter in a three-hour period. The basophil decrease did

not begin until three hours after cortisone and was relatively smaller. In Patients 6 and 8, such a sluggish response of the basophils to prednisone might explain the absence of change in the blood histamine value at three hours.

To attempt to confirm this, studies in Patients 9, 10, and 11 were carried out (Table II).

In Patient 9 the three-hour blood specimen contained more basophils than the control specimen, although the eosinophils were greatly decreased in number. The determined histamine value, however, is less than anticipated on the basis of calculations. Determined and calculated values for the control and three-hour specimens showed a considerable variance, although the determined value of the twenty-four-hour specimen, with a normal number of basophils in the blood, showed good agreement with calculated values. The basophils had diminished in number at four hours but, considering the three-hour counts, exhibited this steroid effect more slowly than the eosinophils. With a single initial dose of prednisone, the histamine blood level at twenty-four hours had even surpassed the control value.

In Patients 10 and 11 the same pattern is evident in the response of basophils and eosinophils to prednisone. A marked reduction in numbers of eosinophils occurred in three hours without much change in the number of basophils. At four hours a reduction in the number of basophils did occur, with a further diminution in eosinophils. In each instance, the lowest actual blood histamine content is found at four hours, together with the lowest basophil counts.

In Patient 11, the discrepancy between calculated and actual histamine values is great, indicating that much less histamine is present in the leukocytes than would be found if they contained the "normal" amount of histamine as established by Graham. This finding emphasizes Graham's observation that the histamine content of a given cell type may be "quite different . . . under different conditions," i.e., in different persons.

In these three patients the changes in blood histamine content compared with relative fluctuations in the numbers of various types of leukocytes also suggest that the ratio of histamine distribution among leukocyte types varies among individual persons. In view of what is known concerning the changing values of other metabolites in various body tissues and the wide range of such values in health and disease, the observed variations in histamine values do not seem surprising. The fate of the disappearing eosinophils and basophils is not known, but their successors promptly appear in comparable number if steroid administration is not continued. Those cells which disappear may contribute to the increase in free urinary histamine which has been found to occur with steroid administration.[10-12] The amount of urinary histamine found under these circumstances approximates that which can be calculated to have disappeared from the blood. One speculates, however, that the kidney must be the site of destruction or removal of these cells, since Adam and his associates[13] have shown that only 1 per cent of free histamine introduced into the plasma is excreted in the urine.

SUMMARY AND CONCLUSIONS

1. In eleven patients studied, most marked changes in leukocytes and in blood histamine content occurred in those patients given moderate amounts of prednisone, contrasted with changes brought about by smaller doses.

2. Fluctuations in blood histamine content parallel more closely the fluctuations in basophil values than fluctuations in other cell types.

3. The histamine content of leukocytes varies in different persons.

REFERENCES

1. Code, C. F.: The Histamine-Like Activity of White Blood Cells, J. Physiol. **90:** 485, 1937.
2. Lowry, O. H., Graham, H. T., Harris, B., Priebat, M. K., Marks, A. R., and Bregman, R. U.: The Chemical Measurement of Histamine in Blood Plasma and Cells, J. Pharmacol. & Exper. Therap. **112:** 116, 1954.
3. Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A., and Parish, H. H., Jr.: Distribution of Histamine Among Leucocytes and Platelets, Blood **10:** 467, 1955.
4. Hills, A. G., Forsham, P. H., and Finch, C. A.: Changes in Circulating Leucocytes Induced by the Administration of Pituitary Adrenocorticotrophic Hormone (ACTH) in Man, Blood **3:** 755, 1948.
5. Nelson, D. H., Sandberg, A. A., Palmer, J. G., and Tyler, F. H.: Blood Levels of 17-Hydroxycorticosteroids Following the Administration of Adrenal Steroids and Their Relation to Levels of Circulating Leucocytes, J. Clin. Invest. **31:** 843, 1952.
6. Code, C. F., Mitchell, R. G., and Kennedy, J. C.: The Effect of Cortisone on the Number of Circulating Basophils and Eosinophils: Is There a Relationship Between These Cells? Proc. Staff Meet., Mayo Clin. **29:** 200, 1954.
7. Ebert, R. H., and Wissler, R. W.: In Vivo Observations of the Effects of Cortisone on the Vascular Reaction to Large Doses of Horse Serum, Using the Rabbit Ear Chamber Technique, J. Lab. & Clin. Med. **38:** 497, 1951.
8. Code, C. F., and Mitchell, R. G.: Histamincytes of the Blood—Eosinophils and Basophils, J. Clin. Invest. **33:** 1924, 1954.
9. Mitchell, R. G.: The Urinary Excretion of Subcutaneously Injected Histamine, Brit. J. Pharmacol. **11:** 462, 1956.
10. Rose, B., Pare, J. A. P., Pump, K. K., Stanford, R. L., Mackenzie, K. R., and Venning, E. H.: Observations of the Metabolic Changes Resulting From the Administration of ACTH to Patients With Asthma and Allied Conditions, Proceedings of the Second Clinical ACTH Conference, Vol. I, New York, 1951, The Blakiston Company.
11. Grob, David: The Renal Excretion of Histamine and Histidine in Man and the Effect of Adrenocorticotrophic Hormone (ACTH) and Cortisone Administration, Bull. Johns Hopkins Hosp. **90:** 341, 1952.
12. Mitchell, R. G., Logan, G. B., Peters, C. A., and Henderson, L. L.: Urinary Excretion of Histamine in Patients Having Asthma and Hay Fever: Observations on Changes Produced by Administration of Cortisone, J. ALLERGY **25:** 504, 1954.
13. Adam, H. M., Card, W. I., Riddell, M. J., Roberts, M., and Strong, J. A.: The Effect of Intravenous Infusions of Histamine on the Urinary Histamine and on Gastric Secretion in Man, Brit. J. Pharmacol. **9:** 62, 1954.

DEVELOPMENT OF HYPERSENSITIVITY TO THIAMINE HYDROCHLORIDE

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APPROXIMATELY 200 cases of allergic or toxic reactions to thiamine have been reported.[1, 2] These reactions have occurred subsequent to intravenous, subcutaneous, intramuscular, or oral administration of the drug.[3-7] Since thiamine hydrochloride has a molecular weight of 337.3, it would not be expected to possess sensitizing capabilities unless spontaneous conjugation with body protein occurred in vivo. Spontaneous conjugation of this type is believed to account for the antigenicity of other medicinal compounds of low molecular weight.[8] This study was made in an attempt to induce hypersensitivity to thiamine hydrochloride experimentally in guinea pigs.

MATERIALS AND METHODS

Albino guinea pigs weighing between 300 and 500 grams, with approximately equal numbers of males and females, were used in the animal experiments. They were maintained on a diet of Rockland guinea pig feed containing 410 to 652 mcg. thiamine per 100 grams.

Thiamine hydrochloride was obtained from two sources: Merck* and Laxseed,† the latter preparation containing 0.5 per cent chlorobutanol. Conjugation was attempted by dissolving thiamine in sterile, normal guinea pig serum to a final concentration of 50 mg. per milliliter and incubating the mixture at 4° C. for twenty-four hours. Adjuvant was prepared as follows: three parts paraffin oil, one part Aquaphor, and one part saline (0.85 per cent sodium chloride) containing 50 mg. thiamine.

Complement-fixation and capillary tube precipitin tests were done in the usual manner. Skin tests were performed by both the intradermal route and the scratch technique suggested by Shapero and Gwinner.[4]

RESULTS

To determine the acute toxicity level of thiamine, twenty-seven normal guinea pigs were inoculated intracardially with from 10 mg. to 25 mg. of the drug and ten additional animals received 50 mg. intra-abdominally (Table I). Although the majority of the animals evidenced some reactions, ranging from transient respiratory distress to generalized weakness and death, no significant gross or microscopic pathologic changes were noted. None of the animals receiving 50 mg. thiamine intra-abdominally evidenced any reaction.

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†Laxseed Company, New York, New York.

Twelve normal guinea pigs received seven intra-abdominal injections of 50 mg. thiamine, administered at four- to six-day intervals. Thirteen days after the last injection, six animals received 5 mg. and six received 10 mg. of thiamine intravenously. The guinea pigs receiving the 5 mg. challenge dose displayed no distress. Of the six receiving the 10 mg. injection, one animal had transient respiratory distress of six minutes' duration, with complete recovery.

Twelve guinea pigs received a single intra-abdominal injection of 50 mg. of thiamine contained in water-oil emulsion, as described under "Materials and Methods." Twelve days later, six of these animals were injected intravenously with 10 mg. of thiamine and only one animal evidenced signs of transient respiratory distress. The remaining six animals were subjected to a series of eight subcutaneous injections of 5 mg. of thiamine at four-day intervals. Eleven days following the last inoculation, the animals were challenged with 10 mg. of thiamine intravenously. The animals exhibited no gross signs of physiologic disturbance and all survived.

TABLE I. THE EFFECT OF INTRACARDIAL OR INTRA-ABDOMINAL INJECTION OF THIAMINE HYDROCHLORIDE IN NORMAL GUINEA PIGS

THIAMINE (MG.)	ROUTE	NO. OF ANIMALS	REACTION* (PER CENT)	DEATH (PER CENT)
10	Intracardial	15	73	6.7
15	Intracardial	4	100	0.0
20	Intracardial	4	100	25.0
25	Intracardial	4	100	50.0
50	Intra-abdominal	10	0.0	0.0

*Reaction is defined as signs of toxicity ranging from transient respiratory distress to generalized weakness of ten to sixty minutes' duration, or death.

Six normal guinea pigs were injected intra-abdominally with 50 mg. of thiamine, incubated in normal guinea pig serum. Challenge of these animals after twelve days with 10 mg. of thiamine intravenously did not elicit an observable reaction. Subsequently, these animals received four intra-abdominal injections of 50 mg. thiamine at weekly intervals, were allowed to rest for ten days, and were then challenged by the intravenous injection of 10 mg. of thiamine. Three of the animals coughed occasionally within five minutes of the injection, but no other symptoms were noted.

Capillary tube precipitin tests on the serum specimens from all experimental animals did not reveal detectable antibodies, although concentrations of thiamine in excess of 5 mg. per milliliter produced a light precipitate with serum from both normal and thiamine-injected animals. Similarly, complement-fixation tests were negative.

Twelve normal animals and twenty-two survivors of the sensitizing experiments were skin tested by both the intradermal route and the scratch technique. The results are shown in Table II. It was apparent that some degree of tolerance to thiamine was evinced in animals having previously received the drug. Scratch tests with the three drug concentrations in both groups of

animals failed to give uniform results. Only four normal and five thiamine-injected animals gave local reactions with the 25 mg. per milliliter preparation.

TABLE II. THE EFFECT OF 0.1 ML. INTRADERMAL INJECTION OF THIAMINE IN NORMAL AND PREVIOUSLY INJECTED GUINEA PIGS

CONCENTRATION OF THIAMINE (MG./ML.)	NORMAL ANIMALS		ANIMALS PREVIOUSLY INJECTED WITH THIAMINE	
	NO.	REACTION* (PER CENT)	NO.	REACTION* (PER CENT)
25.0	12	100.0	22	77.2
2.5	12	41.7	22	0.0
0.25	12	0.0	22	0.0

*Reaction was a rapid appearing, blanched urticarial wheal surrounded by an erythematous area.

DISCUSSION

Although we were able to show the development of a degree of tolerance to thiamine in the guinea pig following repeated injections, we were unable to demonstrate the establishment of a hypersensitive state. We could not demonstrate the formation of an antigenic conjugate of thiamine and serum proteins. Precipitating and complement-fixing antibodies were not demonstrable with the techniques employed.

In 1942, while skin testing human patients, Kalz[9] observed that thiamine was a primary urticariogenic agent. Our observations reaffirm this finding, with the additional observation that guinea pigs possess a higher threshold of reactivity than man. Animals previously injected with thiamine evinced the acquisition of some tolerance by reacting to a lesser degree, or not at all, to concentrations that elicited wheals of fairly consistent diameter in normal animals. The scratch test suggested by Shapero and Gwinner[4] failed to yield uniform results.

SUMMARY

1. We have been unable to demonstrate the development of a hypersensitive state toward thiamine hydrochloride in the guinea pig, employing multiple injections of thiamine, thiamine plus adjuvant, or thiamine incubated with normal guinea pig serum.

2. Precipitating or complement-fixing antibodies could not be demonstrated in animals injected with thiamine, although antibodies similar to those described by Loiseleur and Levy[10] may be present.

3. Multiple injections of thiamine elevated the threshold of skin reactivity of guinea pigs in a manner similar to that described by Haley and Flesher,[11] who used rabbits.

REFERENCES

1. Weigard, C. G.: Reactions Attributed to Administration of Thiamin Chloride, Geriatrics **5**: 274, 1950.
2. Jaros, S. H., Wnuck, A. L., and deBeer, E. J.: Thiamine Intolerance, Ann. Allergy **10**: 291, 1952.
3. Reingold, I. M., and Webb, F. R.: Sudden Death Following Intravenous Injection of Thiamine Hydrochloride, J. A. M. A. **130**: 491, 1946.
4. Shapero, W., and Gwinner, M. W.: Sensitivity to Thiamine Hydrochloride, Ann. Allergy **5**: 349, 1947.

5. Armanino, L. P., and Scott, W. S., Jr.: Anaphylactic Shock Following Intravenous Administration of Thiamine Chloride, California Med. **72:** 178, 1950.
6. Stiles, M. H.: Hypersensitivity to Thiamin Chloride, With a Note on Sensitivity to Pyridoxine Hydrochloride, J. ALLERGY **12:** 507, 1941.
7. Mills, C. A.: Thiamine Overdosage and Toxicity, J. A. M. A. **116:** 2101, 1941.
8. Gell, P. G. H., Harington, C. R., and Rivers, R. P.: The Antigenic Function of Simple Chemical Compounds: Production of Precipitins in Rabbits, Brit. J. Exper. Path. **27:** 267, 1946.
9. Kalz, F.: Thiamine Hydrochloride—An Obligate Wheal Producing Agent, J. Invest Dermat. **5:** 135, 1942.
10. Loiseleur, J., and Levy, M.: La Specificite des Anticorps Consécutifs à L'injection Directe de Molécules Organiques de Faible Paids Moléculaire, Ann. Inst. Pasteur **73:** 116, 1947.
11. Haley, T. J., and Flesher, A. M.: A Toxicity Study of Thiamine Hydrochloride, Science **104:** 567, 1946.

STUDY ON THE EFFECTS OF CORTISONE AND ACTH ON REVERSED ANAPHYLAXIS IN NORMAL AND IRRADIATED ANIMALS

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ARBESMAN, Neter, and Bertram[1] reported in 1951 that ACTH and cortisone protected a certain percentage of guinea pigs from immediate fatal reversed anaphylaxis but gave little protection against active anaphylaxis.

In both reversed and active anaphylaxis, the shock is attributed to the reaction between antigen and antibodies. In active anaphylaxis the shocking reaction is produced by injection of the antigen, while in reversed anaphylaxis antibodies are injected. These antibodies apparently react with antigenic material which is normally present in certain cells and tissues of the animal. Active and reversed anaphylaxis in the guinea pig differ markedly in symptomatology, pathology, and response to antihistaminic drugs.[2, 3]

The purpose of this study was to repeat with some modifications the work of Arbesman and coauthors and, in addition, to study the effect of whole body irradiation on reversed anaphylaxis in normal guinea pigs. Studies in normal and irradiated rats pretreated with ACTH and cortisone were carried out for comparative purposes. In the following report results show that whole body irradiation significantly reduces the protection against immediate reversed anaphylactic shock afforded by cortisone in the normal guinea pig.

METHODS

Anti-guinea pig rabbit sera were prepared as follows: Rabbits were injected intravenously with 2.0 ml. of normal guinea pig serum three times weekly for three weeks. Ten days after the last injection the animals were bled and the precipitin titers of the rabbit antisera were determined. The minimal amount of these antisera which, when injected intravenously or intracardially, produced immediate fatal reversed anaphylactic shock in at least five-sixths of the guinea pigs was determined for each antiserum before each series of experiments.

Forssman antibodies were prepared in rabbits by intravenous injections of 2.0 ml. of a 2 per cent suspension of washed sheep red blood cells three times weekly for three weeks. Following a rest period of ten days, a second series of injections according to the same schedule was given for a period of two weeks. The animals were bled after the last injection. The sera were titrated for sheep

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red-cell hemolysins. The sera with the highest titers were used for the induction of reversed anaphylaxis in guinea pigs. A total of 122 normal hybrid guinea pigs were challenged as controls with these two types of antisera. Injection of equivalent amounts of normal rabbit serum failed to produce anaphylactic shock.

Similarly, anti-rat sera were prepared by injecting rabbits with rat serum. These sera were likewise titrated to find the antisera with the highest anti-rat serum precipitins. Using the sera so selected, the minimum dose (usually 3.0 ml.) needed to induce immediate reversed anaphylaxis was determined for each antiserum before each series of experiments. Thirty rats were thus challenged as control animals in subsequent experiments. Ten rats injected with normal rabbit serum were unaffected. In these experiments rats of the Wistar strain were used. Since no differences were noted in preliminary tests, animals of both sexes were used in the control and experimental groups.

In the irradiation studies the guinea pigs were exposed to approximately an LD-35 dose of ionizing whole body x-irradiation. The irradiation factors were as follows: 250 kv., 15Ma., aluminum parabolic filter (14 mm. thick in center) plus 0.50 mm. copper, target-skin distance 39 inches, rate 7.9 r per minute, total dose 160 r. For rats the factors were the same except that the target-skin distance was 25 inches, the rate 18 r per minute, and the total dose 650 r. Seven days after irradiation the animals were challenged with previously assayed antisera capable of producing immediate reversed anaphylaxis. This timing was selected because some of the effects of irradiation at that time were thought to bear resemblance, to some extent, to the anaphylactic state (prolonged clotting time, drop in platelets, lymphocytes, and polymorphonuclear leukocytes, and elevation in the sedimentation rate).

Guinea pigs were injected by intracardiac puncture, rats through a tail vein. Guinea pigs showing immediate fatal anaphylactic shock were autopsied to rule out trauma as a possible cause of death. Equal numbers of guinea pigs were challenged with antisera containing anti-guinea pig antibodies and with sera containing Forssman antibodies.

Guinea pigs and rats were challenged with injections of antisera after pretreatment with intramuscular or intraperitoneal injections of cortisone. Some guinea pigs were given 25 mg. of cortisone, others 50 mg. This represented a dose of approximately 65 to 130 mg. per kilogram of body weight. These doses were given to groups of animals one, two, three, or twenty-four hours before the challenge with injections of antisera. Rats were given 50 mg. of cortisone (200 mg. per kilogram of body weight) four hours before challenge with antisera.

Guinea pigs and rats were challenged similarly twenty-four hours after an intramuscular injection of 4 mg. of ACTH. Fifty per cent of the guinea pigs in the group pretreated with cortisone were challenged with injections of antisera containing Forssman antibodies, while the others of this group, as well as all those pretreated with ACTH, were challenged only with antisera containing anti-guinea pig antibodies.

Guinea pigs and rats that had been exposed to whole body ionizing irradiation seven days previously, as described above, were challenged with antisera injections four hours after an intramuscular injection of 50 mg. of cortisone. Some guinea pigs, similarly irradiated, were challenged seven days later, twenty-four hours after an intramuscular injection of 4 mg. milligrams of ACTH. The guinea pigs in this experiment were challenged with anti-guinea pig sera only.

RESULTS

The results of these experiments are shown in Tables I and III. Table II summarizes the results of statistical analyses of differences in survival rates of the experimental groups.

TABLE I. INCIDENCE OF IMMEDIATE FATAL ANAPHYLACTIC SHOCK IN THE VARIOUS EXPERIMENTAL GROUPS OF GUINEA PIGS AND RATS

GROUP	TOTAL IN GROUP	NUMBER WITH IMMEDIATE SHOCK AND DEATH	NUMBER RECOVERING AFTER CHALLENGE
Controls			
Guinea pigs	122	115 (94%)	7 (6%)
Rats	30	25 (83%)	5 (17%)
Pretreatment with Cortisone			
Guinea pigs	114	52 (45%)	62 (55%)
Rats	7	7 (100%)	0 (0%)
Pretreatment with ACTH			
Guinea pigs	54	32 (59%)	22 (41%)
Rats	8	8 (100%)	0 (0%)
7 Days Postirradiation			
Guinea pigs	19	16 (84%)	3 (16%)
Rats	16	16 (100%)	0 (0%)
Pretreatment with Cortisone 7 Days Postirradiation			
Guinea pigs	37	34 (92%)	3 (8%)
Rats	10	10 (100%)	0 (0%)
Pretreatment with ACTH 7 Days Postirradiation			
Guinea pigs	45	30 (67%)	15 (33%)
Rats	0	--	--

The data of Table I show that immediate fatal anaphylaxis occurred in 94 per cent of the control group of guinea pigs and in 83 per cent of the control group of rats.

Pretreatment with cortisone significantly lowered the fatal shock rate in guinea pigs from 94 to 45 per cent. Similarly, pretreatment with ACTH significantly lowered the fatal shock rate in guinea pigs from 94 to 59 per cent. A slight increase in shock rate in rats is noted.

While whole body irradiation administered seven days before challenge seems to increase the shock rate in rats and reduce it in guinea pigs, such changes are not statistically significant.

Pretreatment with cortisone seven days after whole body irradiation failed to lower the fatal shock rate in guinea pigs significantly.

TABLE II. STATISTICAL ANALYSES OF DIFFERENCES IN SURVIVAL RATES OF THE EXPERIMENTAL GROUPS

GROUPS COMPARED	CHI SQUARE	p	SIGNIFICANT DIFFERENCE
Guinea pigs			
Controls vs. animals receiving cortisone before challenge	77.70	.01	+
Controls vs. animals receiving ACTH before challenge	33.32	.01	+
Controls vs. irradiated animals	3.30	.10	–
Irradiated animals pretreated with cortisone vs. nonirradiated animals pretreated with cortisone before challenge	24.40	.01	+
Irradiated animals pretreated with ACTH vs. nonirradiated animals pretreated with ACTH before challenge	0.58	.10	–
Rats			
Controls vs. irradiated animals	4.14	.05	–
Rats vs. guinea pigs			
Control rats vs. control guinea pigs	3.96	.02	+
Irradiated rats vs. irradiated guinea pigs	1.22	.10	–

Pretreatment with ACTH seven days after similar irradiation resulted in a significant lowering in the fatal shock rate in guinea pigs from 94 to 67 per cent.

Table III illustrates the effect of timing in pretreatment of guinea pigs with cortisone before challenge with antisera. It is possible that the minor difference in relative effectiveness of cortisone protection in this study, when compared to the results of Arbesman and associates,[1] is related to the increased dosage used and to variations in timing of cortisone injections before challenge.

TABLE III. INFLUENCE OF DOSAGE AND TIME OF ADMINISTRATION OF CORTISONE TO GUINEA PIGS BEFORE ANAPHYLACTIC CHALLENGE

NUMBER OF HOURS BEFORE CHALLENGE CORTISONE GIVEN	DOSE (MG.)	TOTAL IN GROUP	NUMBER DYING IN SHOCK	NUMBER SURVIVING CHALLENGE
1	50	19	16 (84%)	3 (16%)
	25	18	9 (50%)	9 (50%)
	50	25	10 (40%)	15 (60%)
	75	5	4 (80%)	1 (20%)
2	150	4	3 (75%)	1 (25%)
3	50	37	6 (16%)	31 (84%)
24	50	6	3 (50%)	3 (50%)
Totals		114	51 (45%)	63 (55%)

DISCUSSION

The data in this study suggest that: (1) intramuscular injections of cortisone protect nonirradiated guinea pigs from immediate fatal reversed anaphylaxis, (2) cortisone injections do not protect irradiated guinea pigs similarly challenged, and (3) ACTH protects both irradiated and nonirradiated guinea pigs.

Possible explanations for the first two observations may be as follows: Irradiation may depress spontaneous adrenal response to stress. It may cause damage to adrenal glandular cells, as was suggested by Edelmann.[4] In experiments on rats, Edelmann[5] also found that irradiation under proper circumstances

necessitated higher doses of adrenal corticosteroids to protect against stress than in nonirradiated animals. It is to be noted that the doses of cortisone used in this study far exceed any comparable dose per unit of body weight normally used in human beings. Finally, the time-dose relationship in the individual or group of experimental animals cannot be controlled in relationship to the absorption and rate of utilization of the cortisone in these experiments.

The protective effects of ACTH on irradiated guinea pigs may possibly be explained if one assumes that ACTH overcomes the depressive effect of irradiation on the spontaneous adrenal response to stress. It is also possible that the protective adrenal steroid produced in the guinea pig in response to ACTH injections differs in specificity or potency from cortisone given parenterally.

The apparent differences in the rate of immediate reversed anaphylactic fatal shock in guinea pigs and rats are probably due to species differences. Reversed anaphylaxis in rats is induced less readily with some antisera than with others and is less consistently produced than in guinea pigs. This may be the reason that only one previous report[6] of reversed anaphylaxis in rats has appeared in the American literature in the past five years.

CONCLUSIONS AND SUMMARY

The effect of cortisone and ACTH on immediate fatal reversed anaphylaxis was studied in guinea pigs and rats, with and without whole body irradiation. The findings, in general, corroboate the findings of Arbesman, Neter, and Bertram[1] that cortisone and ACTH protect guinea pigs from immediate fatal reversed anaphylaxis, depending on the dose and time of administration of the hormones. Cortisone and ACTH failed to protect rats.

Whole body irradiation did not significantly alter the shock rate in either species.

Following whole body irradiation, cortisone failed to protect against fatal reversed anaphylaxis in both species. ACTH did protect guinea pigs but failed to protect rats seven days postirradiation.

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REFERENCES

1. Arbesman, C. E., Neter, E., and Bertram, L. F.: The Effect of ACTH and Cortisone on Certain Immunologic Mechanisms Including Reversed Anaphylaxis, J. ALLERGY **22**: 340, 1951.
2. Arbesman, C. E., Neter, E., and Becker, C.: The Effect of Pyribenzamine, Neohetramine, and Rutin on Reversed Anaphylaxis in Guinea Pigs, J. ALLERGY **21**: 25, 1950.
3. Hahn, F., and Oberdorf, A.: Antihistaminica und Anaphylaktoide Reaktionen, Ztschr. Immunitätsforsch. u. exper. Therap. **107**: 528, 1950.
4. Edelmann, Abraham: Adrenal Shielding and Survival of Rats After Irradiation, Am. J. Physiol. **165**: 57, 1951.
5. Edelmann, Abraham, and Katsch, Seymour: Survival of Rats Adrenalectomized After X-irradiation, Am. J. Physiol. **168**: 626, 1952.
6. Rothbard, Sidney, and Watson, Robert R.: Antigenicity of Rat Collagen; Reversed Anaphylaxis Induced With Anti-rat Collagen Serum, J. Exper. Med. **103**: 57, 1956.

EXPERIMENTAL SENSITIZATION WITH PENICILLINASE

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A RECENT study by Becker[1] described the action of penicillinase on circulating penicillin in vivo in guinea pigs and in human beings. It was found that parenteral administration of penicillinase inactivated all circulating penicillin, both in guinea pigs and in human beings. The therapeutic use of purified penicillinase for inactivation of circulating penicillin in patients suffering allergic reactions to penicillin was suggested.

After repeated injections of penicillinase in guinea pigs neither toxic, hypersensitive, nor anaphylactic responses were observed.[1] However, sensitization of rabbits with penicillinase was reported earlier by Perlstein and Liebmann,[2] as well as by Housewright and Henry.[3] These authors found considerable amounts of specific precipitins in the sera of immunized rabbits.

The purpose of the present study was to re-examine the antigenicity of penicillinase in guinea pigs and rabbits and, if sensitization was produced, to determine the immunologic properties of their sera.

EXPERIMENTAL TECHNIQUES

Antigen.—Injectable penicillinase* is obtained from cultures of *Bacillus cereus,* strain NRRL-B 569. It is a purified, nonpyrogenic grade of enzyme in lyophilized form. Its potency is expressed in Schenley units: 1 unit of penicillinase will inactivate 1 unit of penicillin within one minute and 60 units of penicillin within one hour at 25° C. at a pH of 7.4. Our determinations have shown that 1,000,000 units of penicillinase contain 3 to 5 mg. of phosphotungstic-precipitable nitrogen.

Sensitization.—Male albino guinea pigs, each weighing 350 to 400 grams, and male albino New Zealand rabbits, each weighing 2,500 grams, were used for sensitization.

Group I: Nine guinea pigs and two rabbits were injected with two antigens—penicillinase and human albumin—diluted in saline and blended homogenously into Freund's adjuvant. All animals in this group were given one intramuscular injection of a solution composed of 300,000 units of penicillinase and 10 mg. of human albumin in 0.5 ml. of saline blended with 1 ml. of Freund's adjuvant containing 0.5 mg. of *Mycobacterium butyricum* (Bacto-Adjuvant-Complete, Difco Laboratories, Detroit, Michigan).

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TABLE I

GROUP	GUINEA PIG NUMBER	SENSITIZED WITH	SKIN TESTING WITH PENICILLINASE		IMMUNOLOGIC PROPERTIES OF ANTISERA						
					ANTISERA INDIVIDUALLY			ANTISERA POOLED (EQUAL VOLUMES OF EACH)			
			IMMEDIATE RESPONSE (b)	ARTHUS RESPONSE	PRECIPITATION (c)	PCA IN 1:5 DILUTION (d)		PRECIPITATION (TITER) (e)	AGGLUTINATION (TITER) (f)	PCA (TITER) (g)	PSA (g)	PKR (g)
I	1 (a)	Penicillinase									
	2	Human albumin	+++	Arthus	+++	+++					
	3	Adjuvant	+++	Arthus	++	+++					
	4		++	–	++	++					
	5		+++	Arthus	++	+++		+	+	+	+	–
	6		+++	Arthus	++	+++		(1:320)	(1:160)	(1:3200)		
	7		+	–	+	+	(1:80)					
	8		++	–	+	+++						
	9 (a)											
II	10	Penicillinase	+	–	+	++						
	11	Human albumin	–	–	+	+						
	12		+	–	+	++						
	13		–	–	+	++						
	14		+	–	+	++		+	+	+	+	–
	15		–	–	+	+	(1:10)	(1:10)	(1:20)	(1:200)		
	16		+	–	+	++						
	17		–	–	+	++						
	18 (a)											
III	19	Penicillinase	+	–	+	+++						
	20	Adjuvant	+++	Arthus	+++	+++						
	21		+++	Arthus	+++	+++						
	22		+++	Arthus	+++	+++						
	23		+	–	+	++		+	+	+	+	–
	24		+++	Arthus	+++	+++		(1:1280)	(1:640)	(1:6400)		
	25		+++	Arthus	+++	+++						
	26		+++	Arthus	++	+++						
	27		+++	Arthus	+++	+++						

IV	28	Penicillinase	+	–	+	++						
	29		–	–	+	+ (1:160)						
	30		+	–	+	++						
	31		–	–	+	+ (1:80)						
	32		++	–	++	+++						
	33		+++	Arthus	+++	+++	+ (1:40)	+ (1:80)	+ (1:400)	+	–	
	34		+	–	+	++						
	35		++	–	+	++						
	36		–	–	+	++						

(a) Animal died during experiment.

(b) The intensity of immediate response is represented by basic gradings as follows: (+) erythema-edema after one hour; (++) erythema-edema progressing to infiltration; (+++) erythema-edema with central blanching and progressive infiltration.

(c) Estimates of precipitin content were made on a comparative basis. (+) indicates the weakest precipitation. (++) and (+++) indicate a stronger precipitation and the strongest, respectively; precipitation determined by agar gel diffusion.

(d) All antisera were individually tested for PCA in 1:5 saline dilutions. The PCA reactions were estimated on a comparative basis. The relatively weakest reactions were classified as (+) (blue effusion of 7 to 15 mm. in diameter). Antisera responsible for (+) reaction were re-examined in PCA end point titration experiments. PCA titers of these antisera are included in the table.

(e) Classical test tube method of precipitation (antibody dilutions: antigen) was used.

(f) The technique of increasing antibody dilutions and adding an equal amount of antigenic particles was employed. Pooled serum of normal guinea pigs used in control agglutinations, agglutinated *B. cereus* in 1:10 dilution.

(g) Abbreviations: PCA = Passive cutaneous anaphylaxis; PSA = Passive systemic anaphylaxis (shock); PKR = Prausnitz-Küstner reaction (passive sensitization of human skin).

This injection was followed for a period of two weeks by semiweekly intramuscular administration of the same quantities of the two antigens in 0.5 ml. of saline blended with 0.5 ml. of Freund's adjuvant without mycobacteria (Bacto-Adjuvant-Incomplete, Difco Laboratories).

Group II: Nine guinea pigs and two rabbits were injected with two antigens, as in Group I, but without Freund's adjuvant.

Group III: Nine guinea pigs and two rabbits were injected with penicillinase only; 300,000 units of penicillinase in 0.5 ml. of saline were mixed with 1 ml. of Freund's adjuvant with mycobacteria. The initial injection was followed by semiweekly intramuscular administration of the same amount of penicillinase in 0.5 ml. of saline and 0.5 ml. of Freund's adjuvant without mycobacteria, over a period of two weeks.

Group IV: Nine guinea pigs and two rabbits were injected with penicillinase in saline (as in Group III) but without Freund's adjuvant.

Skin Testing.—Seven days before bleeding, all of the sensitized animals were tested by intradermal injection of 25,000 units of penicillinase in 0.1 ml. of saline. (Skin tests on normal animals failed to produce reactivity upon injection of the same and double the concentration of penicillinase.) The reactions were read several times within forty-eight hours after testing and were considered positive if, within the first hour, an erythema and edema developed at the tested sites. If this immediate reaction progressed to central blanching, infiltration, and central necrosis, the reactions were recorded forty-eight hours later as Arthus's phenomena.

Sera.—Three weeks after completion of the injection course, and seven days after skin testing, 10 ml. of blood was withdrawn from each animal and the sera were individually tested for precipitins and anaphylactic reactivity. Then equal volumes of the sera obtained from each of the animals were pooled by groups and the pooled sera were tested by quantitative immunologic procedures for precipitins, agglutinins, anaphylactic property (guinea pig smooth muscle-sensitizing property),[6] and human-skin-sensitizing property (Table I).

Passive Cutaneous Anaphylaxis in Guinea Pig (PCA)[6].—Male albino guinea pigs, weighing 350 grams each, were used. Shaved abdominal skin was employed for passive transfer of 0.1 ml. per site of antisera or antisera dilutions in 0.85 per cent saline. Six hours later an intracardial challenge with a mixture of 300,000 units of penicillinase and 1 ml. of 1 per cent Evans blue was given. The antiserum-saline dilution responsible for a minimal (7 mm. diameter) effusion of dye established the titration end point, that is, the maximal dilution of antibody capable of inducing a PCA reaction. The titration by dilution permitted the estimation of the sensitizing potency of the investigated sera and quantitative comparisons.

Passive Systemic Anaphylaxis (PSA).—Passive systemic sensitization of guinea pigs was accomplished by an intraperitoneal injection of 5 cm. of sensitive serum; twenty-four hours later the animals were challenged intravenously with 300,000 units of penicillinase. The PSA was considered positive if anaphylactic dyspnea ended in death and autopsy revealed emphysematous lungs.

Precipitation.—The technique described as agar gel diffusion Oudin[7] and the classical test tube method were used. For the test tube method, sera were diluted twofold in series with saline, and an equal amount of antigen (100,000 units penicillinase in 0.1 ml.) was added to each test tube. The mixture was incubated for two hours at 37° C. and at 5° C. overnight. Results were read the following day.

Agglutination.—To increasing dilutions of serum was added an equal volume of a suspension of washed *Bacillus cereus,* grown on Bacto-brain-heart infusion broth and dextrose medium containing 10 international units of penicillin per one milliliter.[8] The mixture was incubated for two hours at 37° C. and at room temperature overnight. Results were read the following day.

Prausnitz-Küstner Reactions (PKR).—Seitz-filtered sera, undiluted and diluted in 0.85 per cent saline, were injected intracutaneously in a volume of 0.1 ml. in normal subjects. The skin sites were challenged with 2,500 units of penicillinase in 0.05 ml. of saline forty-eight hours later.

Controls.—Parallel tests with normal rabbit and guinea pig sera served as controls. These were negative except that agglutination of *Bacillus cereus* occurred with both normal guinea pig and normal rabbit serum in dilutions through 1:10 but not higher.

RESULTS AND DISCUSSION

Table I shows the result of an attempt to sensitize thirty-six guinea pigs with penicillinase. All animals became sensitive. This is demonstrated by various immunologic tests. The Freund adjuvant markedly potentiated the sensitization as shown by the results of skin testing and by the increase in antipenicillinase precipitins, agglutinins, and anaphylactic reactivity (PCA). Although seven out of sixteen guinea pigs injected without adjuvant failed to reveal any skin reaction, antipenicillinase precipitins (determined by the agar gel diffusion technique) and anaphylactic reactivity (determined by PCA and PCA end point titrations) were noted in their sera.

All the antisera of the guinea pigs which were injected with both penicillinase and human albumin contained precipitins and anaphylactic antibody against both antigens. Pooled antisera of these animals induced PCA reactivity to albumin in dilutions 1:1,600 (Group I) and 1:100 (Group II).

Eight rabbits injected by the same technique as guinea pigs became sensitive to penicillinase as judged by tests for precipitins, agglutinins, anaphylactic reactivity, and human-skin-sensitizing property. The titer of antibodies in the rabbits' pooled sera was found to be higher than in the guinea pigs' pooled sera. (For instance, the PCA titers in the rabbits' pooled sera was as high as 1:8,000 for Group I, 1:1,000 for Group II, 1:16,000 for Group III, and 1:1,000 for Group IV).

Contrary to the negative results of passive sensitization of human skin obtained with guinea pig antisera, the sera of two rabbits transferred sensitivity to human skin in dilutions of 1:40 and 1:20. The capacity of these two

sera to induce human skin sensitivity was not proportional to their capacity to induce PCA, precipitation, and agglutination (as reflected by PCA, precipitation, and agglutination titers).

SUMMARY

Thirty-six guinea pigs and nine rabbits were given five semiweekly injections of penicillinase. Some animals received pure penicillinase, whereas others received it in combination with human albumin and/or Freund's adjuvant. All of the animals became sensitive to penicillinase. The degree of sensitivity was measured quantitatively by determining levels of circulating precipitins, agglutinins, and anaphylactic reactivity. Freund's adjuvant potentiated the sensitizing activity of penicillinase.

REFERENCES

1. Becker, R. N.: Effect of Penicillinase on Circulating Penicillin, New England J. Med. **254:** 952, 1956.
2. Perlstein, D., and Liebmann, A. J.: The Production of Anti-Penicillinase Immune Sera, Science **102:** 197, 1945.
3. Housewright, R. D., and Henry, R. J.: Studies on Penicillinase. III. The Effect of Antipenicillinase on Penicillin-Resistant Organisms, J. Bact. **53:** 241, 1947.
4. Levy, B. G.: A Unit of Penicillinase, Nature, London **166:** 740, 1950.
5. Freund, J., and McDermott, K.: Sensitization to Horse Serum by Means of Adjuvants, Proc. Soc. Exper. Biol. & Med. **49:** 548, 1942.
6. Fisher, J. P., and Cooke, R. A.: Passive Cutaneous Anaphylaxis (PCA) in Guinea Pig, J. ALLERGY **28:** 150, 1957.
7. Munoz, J., and Becker, E. L.: Antigen-Antibody Reaction in Agar, J. Immunol. **65:** 47, 1950.
8. Shenley Laboratories, Inc., New York, N. Y.: Personal communication.

THE ROLE OF ANTICOAGULANT SUBSTANCES (DICUMAROL AND HEPARIN) IN THE PREVENTION OF THE LOCAL SHWARTZMAN REACTION

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THE formation of thrombi in the small blood vessels of the skin is an essential feature in the development of the local Shwartzman reaction. Gerber,[3] in 1936, described thromboses of the cutaneous veins, consisting of amorphous acidophilic masses mixed with scattered leukocytes, erythrocytes, and "little fibrin," chiefly at the periphery of the thrombus. Stetson[10, 11] observed the formation of leukocyte-platelet thrombi within the capillaries and veins one hour after the "provoking" injection. The vasoconstriction, which is found at the earliest stages of the reaction, supposedly favors the thrombus formation by slowing the blood flow; approximately four hours after the provocative injection, necroses of the thrombosed vessels occur and hemorrhages appear around these blood vessels, whose walls have become necrotic in the later course of the process.

The inhibition of the local Shwartzman reaction by high doses of heparin[2, 6] seems to be directly related to the anticoagulant activity of this substance, but experimental proof is lacking so far as to whether other pharmacological properties of heparin might also play a role. Anticoagulant substances of the Dicumarol group gave contradictory results in the hands of different authors.

Rothfeld and associates failed to observe an inhibiting action of Dicumarol on the local Shwartzman reaction. Good[5] found an inhibiting action of tromexan, and Spanoudis, Eichbaum, and Rosenfeld[9] reported prevention of the phenomenon by high doses of Dicumarol, a result which was confirmed by a newer experimental series to be dealt with in this article.

This newer series comprises fourteen rabbits treated with Dicumarol, three rabbits treated with heparin, eleven normal controls and one normal rabbit which received only the sensitizing (intracutaneous) dose of Shear's *Serratia marcescens* toxin.

There was a positive Shwartzman reaction in all normal controls. Of the animals which had been treated with Dicumarol, eleven gave a negative reaction; two more rabbits showed a "precocious" hemorrhage (that is, a local subepithelial hemorrhage after sensitization only), which was followed by a fully developed positive local Shwartzman reaction; a third animal gave a doubtful reaction. The treatment with heparin prevented the local Shwartzman reaction in two animals; a third one showed a weakly positive Shwartzman phenomenon.

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The special purpose of these experiments was to study the mechanism by which the anticoagulants interfere with the local Shwartzman reaction.

MATERIAL AND METHODS

Rabbits of either sex, weighing 2,000 to 3,000 grams, received an intradermal injection of Shear's *Serratia marcescens* toxin at four to six sites on the depilated abdominal skin; 0.04 mg. of toxin, contained in 0.1 ml. saline, was injected at each of these sites, which lay at least 4 cm. from each other. The provoking intravenous injection of 0.1 mg. of toxin in 2 ml. saline was given twenty-four hours later.

Skin fragments for histologic examination were taken twenty-four hours after sensitization and two, twenty-four, and forty-eight hours after provocation. For this purpose, the skin was lifted with a pincette at the sites of cutaneous sensitization and clipped away with large thin-bladed scissors. The gaping wound was closed with surgical Michel clamps. No significant hemorrhage or infection occurred, even in the heparinized or Dicumarol-treated animals. The skin fragments thus obtained were fixed in a 10 per cent formalin solution and, after embedding in paraffin, were dyed with hematoxylin and eosin, Weigert's fibrin, van Gieson's, and phosphotungstic-hematoxylin stains.

This method eliminates the variation of individual factors by studying the histologic evolution of the Shwartzman phenomenon at various stages in the same animal. Even under these circumstances, however, it sometimes occurred that in one and the same animal the intensity of the reaction was not equally strong at all injected places, which in my opinion must be related to lesser technical differences, such as injection of slightly different amounts of toxin, deposition of toxin into deeper or higher skin layers, etc.

Details of the treatment with Dicumarol, which reduced the prothrombin level to less than 1 per cent, are described in an earlier paper.[9]

The animals which had been treated with heparin received three times 30 mg. of this substance, at the time of provocation and two and four hours afterward.

The clotting time four hours after provocation was, in all cases, longer than two hours (Lee-White method).

RESULTS

I. *Controls.*—The histologic analysis of the local Shwartzman reaction in normal control animals showed three principal stages: (1) the stage of inflammation, (2) the stage of primary hemorrhage, and (3) the stage of hemorrhagic necrosis.

1. *The first stage* (*stage of inflammation*) develops within the twenty-four hours that follow intracutaneous sensitization. The intensity of the inflammatory reaction found at this moment varies greatly with different animals and has no direct relation to the degree and extension of the following Shwartzman

reaction which appears after intravenous provocation. The inflammatory reaction is characterized by an edema of the subpapillary skin layers and accumulation of white cells in the interstitium, principally of polymorphonuclear neutrophils, "pseudo-eosinophils,"[11] and fewer round cells. These leukocytes may be scattered, without apparent order, from the subepithelial layers down to the subcutis. Sometimes they seem to follow, like a shoal of fish, the smaller lymph vessels, or eventually they may form the characteristic "cuffs" around the capillaries and smaller veins. Also inside these vessels one can observe a striking increase of white cells, which sometimes might reach such a degree that they literally form a plug within the capillaries. Occasionally one encounters a swelling of the capillary endothelial cells. The interstitial histiocytes are swollen and more numerous than normal. There is only a moderate hyperemia of subepithelial and subcutaneous veins. A small epithelial hemorrhage at this stage of the process occurred only twice in eleven observations; in both cases, the described inflammatory reactions were unusually strong. None of the other nine cases in this group showed such a "precocious" hemorrhage.

2. *The second stage* (*stage of "primary hemorrhage"*) develops within the first two hours following the provocative toxin injection. This stage is characterized by the appearance of an extensive subepithelial hemorrhage in addition to the described inflammatory reactions, which now are generally more pronounced than at the first stage. We call this hemorrhage, which apparently occurs without any gross anatomic lesion of the vessel wall, "primary hemorrhage." There is an intense vasodilatation of subepithelial and subcutaneous veins and capillaries, but no red or mixed thrombi can be found anywhere. White thrombi, consisting of leukocytes and amorphous masses (platelets), are seen at various sites in the inflamed area, within the capillaries and small veins, but definitely not with the frequency observed by Stetson,[10] who states, "Nearly all of the small veins and capillaries show obliteration by cellular plugs or thrombi." It seems worth mentioning that the same leukocyte-platelet thrombi occurred occasionally also at the first stage, that is, twenty-four hours after sensitization and still *before* the application of the provoking toxin dose. No significant changes could be observed on either arteries or arterioles.

3. *The third stage* (*stage of hemorrhagic necrosis*) develops within the three to twenty-four hours that follow the provocative toxin injection. This stage is characterized by extensive formation of red and mixed thrombi in the capillaries and the smaller and medium-sized veins, especially in the upper parts of the subcutis, and by necroses of the vessel walls, karyorrhexis and karyolysis of the interstitial leukocytes and round cells (leukoclasia), with formation of sterile abcesses. The mural necrosis of the thrombosed veins occurs initially at a smaller section of the circumference, where the endothelial cells appear swollen and the muscular layers become invaded by polymorphonuclear leukocytes; gradually, the entire wall structures are destroyed and the blood floods through the ruptured vessel wall into the surrounding inflamed area. Contrary to the "primary hemorrhage" at the second stage, which is due to the diapedesis through the intact vessel wall, we call this hemorrhage through the ruptured necrotic

vessel wall "secondary hemorrhage." Forty-eight hours after provocation, but sometimes after twenty-four hours and possibly even earlier, this blood-infarcted area becomes necrotic in its whole extension from the upper epithelial layers down to the subcutaneous tissue, involving sometimes even the deeper fascial and muscular layers. Occasionally, within this infarcted zone one also observes completely necrosed arteries and arterioles. The transition from the necrotic to the neighboring normal tissue occurs with a striking suddenness; the normal tissue at the very border of the necrotic zone usually shows only a weak inflammatory reaction.

This description of the local Shwartzman reaction in normal control animals does not differ essentially from the earlier observations by Gerber[3] in 1936 and Stetson[11] in 1952.

II. *Dicumarol Treatment.*—In "Shwartzman-negative" rabbits which had been treated with Dicumarol, the stage of inflammation (Stage 1) was generally very weak when compared with the average picture of Shwartzman-positive control animals.

At Stage 2, that is, two hours after the provoking toxin injection, the histologic picture differed from the normal controls in one principal point: the subepithelial hemorrhage which normally characterizes this stage was completely lacking in these Shwartzman-negative animals. Otherwise, the inflammatory cellular and vascular changes were almost identical in the Dicumarol-treated animals and the control group, although a certain tendency to weaker reactions still seemed to prevail in the former group. Intracapillary leukocyte-platelet plugs occurred with about the same frequency in both groups.

At Stage 3, that is, twenty-four hours after the provoking injection, the inflammatory reactions persisted to a slighter or stronger degree, but no hemorrhages, thrombi, or necroses could be observed anywhere.

III. *Heparin Treatment.*—The Shwartzman-negative rabbits which had been treated with heparin showed almost the same picture as the Dicumarol-treated animals at Stages 2 and 3: complete absence of hemorrhagic, thrombotic, or necrotic lesions and weak inflammatory reactions.

In those cases in which the Dicumarol or heparin treatment failed to prevent the local Shwartzman reaction, the histologic picture gave no principal difference from normal controls, although hemorrhage and necrosis seemed generally more limited in animals which had been treated with the anticoagulant substances.

DISCUSSION

It appears from the foregoing description that the subepithelial hemorrhage, which develops within the first two hours after provocation, is the *primum mobile* or at least the first signal of the fully developed local Shwartzman reaction. This "primary hemorrhage" (Stage 2) precedes the formation of red thrombi in capillaries and veins and is inhibited, in most cases, by adequate doses of heparin or Dicumarol.

In absence of histologic evidence of any ruptured or otherwise damaged vessels, the "primary hemorrhage" can be explained only by diapedesis through the intact vessel wall.[1] This, however, gives no clue as to the inhibiting action of the anticoagulants at this stage of the process (Stage 2) where no vascular thrombi can be found as yet. Therefore, the specific antithrombotic activity of Dicumarol or heparin cannot interfere with this primary hemorrhage and should influence only thrombus formation and secondary hemorrhage at Stage 3.

The crucial point, however, is the prevention of the primary hemorrhage. The following hypothesis is suggested to explain the inhibitory activity of the anticoagulants at this early stage of the local Shwartzman reaction.

The fundamental vascular changes, namely, leukocyte-platelet plugs and extreme vasodilatation with slowing of local blood circulation and possibly formation of blood sludges have the effect of a functional thrombus by inhibiting a sufficient afflux of blood; this causes a relative anoxemia of the tissue, which might result in an altered capillary permeability.[12, 13] Vasoconstriction (early phase) and stagnation of blood in maximal dilated vessels (late phase) should affect the local blood supply in a similar manner, namely, by decrease of optimal oxygen concentration. Since in the local Shwartzman reaction the intravascular accumulation of platelets is a regular event, especially at the early stages of the reaction, the vasoconstriction due to a substance liberated from disintegrating platelets[15] might possibly contribute also to the observed narrowing of blood vessels.*

Heparin, although it does not prevent sludge formation, inhibits its adhesion to the vessel wall and thus prevents the further development of a thrombus.[4] This is due to the particular property of heparin of diminishing the tendency toward platelet agglutination and disintegration. The adhesiveness of platelets is likewise inhibited by Dicumarol.[8] By this mechanism, the sticking of leukocyte-platelet or erythrocyte plugs to the vessel wall is prevented, the interruption of the blood flow will not be permanent, and the vessel wall is less injured. There would be no diapedesis of red blood cells through the intact capillaries ("primary hemorrhage"); nor would there be thrombus formation, necrosis, or rupture of the vessel wall ("secondary hemorrhage").

There is still another property of heparin, its antienzymatic (that is, its antiproteolytic and hyaluronidase-inhibiting) activity, which also might play a role in the prevention of the local Shwartzman phenomenon by this substance. The activation of tissue proteases in the local Shwartzman reaction[10] might be slowed down by heparin. An analogous activity of Dicumarol has not been reported as yet, to my knowledge. The weak inflammatory reaction in our Dicumarol-treated animals, with a negative Shwartzman phenomenon, might also be interpreted as a sign of a possible anti-inflammatory activity of this drug.

One fact cannot be dismissed without comment, to wit, that a certain, yet small, percentage of heparin- and Dicumarol-treated animals gave a positive local Shwartzman reaction, although clotting time and prothrombin level, respectively, equalled that of their Shwartzman-negative similars. (See also Good and

*In view of the recent discoveries, one might think of serotonin or like substances.

Thomas.[6]) In all these cases the clotting factors were changed to such a degree that thrombus formation should have been inhibited but, nevertheless, a positive reaction occurred which cannot be explained satisfactorily.

In conclusion, I believe that the inhibiting action of heparin and Dicumarol on the local Shwartzman phenomenon is only partly due to their anticoagulant effect. It is suggested that other properties of these substances, such as prevention of platelet agglutination and lysis, as well as their antienzymatic activity, might play at least a supplementary role.

SUMMARY

Three stages are distinguished in the histologic evolution of the local Shwartzman reaction:

(1) Stage of inflammation (twenty-four hours after sensitization).

(2) Stage of "primary" hemorrhage (two hours after provocation)—diapedesis bleeding through the intact capillary vessels into the subepithelial skin layers. At this stage no red or mixed thrombi can be found in the blood vessels.

(3) Stage of hemorrhagic necrosis (three to twenty-four [forty-eight] hours after provocation)—intravascular thrombosis, necrosis of vessel wall, "secondary" hemorrhage through the ruptured vessel wall, hemorrhagic infiltration and necrosis (infarction) of the neighboring cutaneous tissue.

Leukocyte-platelet thrombi were found in our series, not only after provocation, but also after simple sensitization and even in Shwartzman-negative animals that had been treated with anticoagulants.

Our findings that hemorrhage (Stage 2) precedes thrombus formation are in agreement with earlier observations by Gerber.[3]

In Dicumarol- or heparin-treated rabbits with negative Shwartzman reaction no primary hemorrhage (Stage 2) or hemorrhagic necrosis (Stage 3) appeared, although otherwise the local inflammatory reactions were roughly identical to those observed in positive control animals.

The inhibition of the primary diapedesis hemorrhage cannot be due to the anticoagulant property of these drugs, whose activity should be expected only at a stage where thrombus formation occurs, that is, between Stage 2 and the fully developed Stage 3.

It is suggested, therefore, that other pharmacologic properties of these drugs, such as inhibition of platelet agglutination and lysis, as well as their antienzymatic activity, also play an important role in the suppression of the local Shwartzman reaction.

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REFERENCES

1. Apitz, K.: Die Wirkung bakterieller Kulturfiltrate nach Umstimmung des gesamten Endothels beim Kaninchen, Virchows Arch. f. path. Anat. **293:** 1-33, 1934.
2. Cluff, L. E., and Berthrong, M.: The Inhibition of the Local Shwartzman Reaction by Heparin, Bull. Johns Hopkins Hosp. **92:** 353-364, 1953.
3. Gerber, J. E.: Morphologic Aspect of the Local Shwartzman Phenomenon, Arch. Path. **21:** 331-350, 1936.
4. Girner, J.: Observations on Blood Sludging, Am. J. Ophth. **36:** 1063-1067, 1953.
5. Good, R. A., quoted from Thomas, L.: The Physiological Disturbances Produced by Endotoxins, Am. Rev. Physiol. **16:** 467-490, 1954.
6. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction. IV. Prevention of the Local and Generalized Shwartzman Reaction With Heparin, J. Exper. Med. **97:** 871-888, 1953.
7. Rothfeld, B., Williams, C. H., Thaler, W. J., and Rodas, J. M.: Effect of Dicumarol on Localized Shwartzman Reaction, Proc. Soc. Exper. Biol. & Med. **87:** 5-7, 1954.
8. Schmid, J.: Die Blutgerinnung in Theorie und Praxis, Vienna, 1951, Wilhelm Maudrich.
9. Spanoudis, S., Eichbaum, F. W., and Rosenfeld, G.: Inhibition of the Local Shwartzman Reaction by Dicumarol, J. Immunol. **75:** 167-170, 1955.
10. Stetson, C. A., Jr.: Studies on the Mechanism of the Shwartzman Phenomenon; Certain Factors Involved in the Production of Local Hemorrhagic Necrosis, J. Exper. Med. **93:** 489-503, 1951.
11. Stetson, C. A., Jr.: The Pathogenesis of the Shwartzman and Arthus Phenomenon and Their Relation to Human Rheumatic Fever, in Thomas, L. (editor): Rheumatic Fever, a Symposium, Minneapolis, 1952, University of Minnesota Press, pp. 224-231.
12. Stetson, C. A., Jr.: Studies on the Mechanism of the Shwartzman Phenomenon; Similarities Between Reactions to Endotoxins and Certain Reactions of Bacterial Allergy, J. Exper. Med. **101:** 421-436, 1955.
13. Thomas, L., and Stetson, C. A., Jr.: Studies on the Mechanism of the Shwartzman Phenomenon, J. Exper. Med. **89:** 461-477, 1949.
14. Wintrobe, M. M.: Clinical Hematology, ed. 3, Philadelphia, 1951, Lea & Febiger, p. 999.
15. Zucker, M. B.: Platelet Agglutination and Vasoconstriction as Factors of Spontaneous Hemostasis in Normal, Thrombocytopenic, Heparinized and Hypoprothrombinic Rats, Am. J. Physiol. **148:** 275-288, 1947.

STUDIES ON THE ANTIGENICITY OF EGG YOLK

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PEDIATRICIANS are constantly being made aware of the number of infants who react adversely when egg yolk is first introduced into the diet. Symptoms most commonly encountered include refusal of the yolk, regurgitation, skin rashes of the eczematous type, and occasionally respiratory symptoms such as "head colds" or, rarely, asthma. It is generally agreed that skin rashes and respiratory symptoms are due to the antigenic properties of the egg yolk or the egg white which adheres to it in normal kitchen preparation, while gastrointestinal manifestations of egg yolk idiosynerasy may be due to either the antigenic or the physical properties of the food.

The avian egg is a closed system which contains all the protein requirements of a hatched chick. More investigation on the proteins of egg white has been done because of its greater simplicity of composition.

The proteins of egg white, according to Warner,[1] are:

Ovalbumin	69.5%*
Conalbumin	9.0%
Ovomucoid	12.9%
Lysozyme	
Globulin	6.7%
Ovomucin	1.9%
Avidin	Trace

The major proteins of egg yolk occur as lipoproteins and, since the methods for fractionating lipoproteins are poorly developed, there have been isolated

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*Of total protein by chemical methods.

by chemical analysis no proteins comparable in homogeneity to those from egg white, according to Warner who lists the following lipid-free proteins of egg yolk:

Vitellin	24 to 52%, depending on method
Vitellenin	25%
Livetin	22%
Phosvitin	7%

By immunologic methods, proteins common to egg white and egg yolk have been found. Marshall and Deutsch[2] found conalbumin, the albumin of chicken blood serum, present in egg white (16 per cent) and in egg yolk (4 per cent). They found ovalbumin barely detectable in egg yolk, and lysozyme and ovomucoid absent from egg yolk. However, Onoe[3] did isolate a very small quantity of ovomucoid from egg yolk. Fränkel and Jellinek[4] found that a fraction identical with ovomucoid from egg white exists in egg yolk.

Hektoen and Cole[5] have shown by precipitation experiments the presence of five distinct antigens in egg white, namely, ovoglobulin, ovomucin, ovalbumin, conalbumin, and ovomucoid. They found ovomucin and ovomucoid to be weaker antigens than the other three. This confirmed the opinion of Lewis and Wells,[6] who thought that the heat used in the extraction of these two proteins may have decreased their antigenicity.

Coulson and Stevens[7] have demonstrated by the Schultz-Dale technique that there is an antigen (not ovalbumin) which is common to both egg white and egg yolk. It could be any of the other components of egg white or a combination of all of them, but since conalbumin has been found in larger quantities in yolk and is a more potent antigen than ovomucoid or ovomucin, the evidence is in its favor as being the major portion of this common antigen.

The purpose of this study was twofold:

1. To determine the relative antigenicity of raw egg yolk, hard-boiled egg yolk, and a commercial variety of egg yolk* especially prepared for infant feeding.
2. To determine to what extent adherent egg white was responsible for any antigenicity exhibited by egg yolk.

During the course of the study, three experimental approaches were used: (1) the study of passively transferable antibodies in human serum, (2) anaphylactic studies in guinea pigs, and (3) antigenic studies on the uterine horns of sensitized guinea pigs by means of the Schultz-Dale technique.

PASSIVE TRANSFER STUDIES

Sixteen patients with a past or present history of egg sensitivity were selected from children attending the Allergy Clinic of the Children's Hospital

*The commercially prepared egg yolk used throughout the study was furnished by The Gerber Products Company and is readily available as *Gerber's strained egg yolks.* Its preparation is as follows: A homogenized egg slurry is prepared from frozen egg yolks and denaturation is begun by flash heating the slurry to 240° F. The product is then flash cooled, poured into containers, and sealed. After sealing, the product is sterilized by heating to 240° F. for forty-five minutes.

of the District of Columbia. These were selected because they gave marked skin test reactions to egg yolk, although all gave positive reactions to egg white as well. Eight of the sixteen patients had both eczema and asthma, while the other eight had either eczema or asthma.

Eight cubic centimeters of venous blood was drawn from each of these patients, and after clotting had occurred the serum was separated and passed through a Seitz filter. The resulting serum was cultured for sterility, and syphilis was ruled out by a cardiolipin microflocculation test. One and one-half cubic centimeters of serum was incubated with 1,000 protein nitrogen units of egg white for twenty-four hours in a refrigerator for neutralization of egg white antibodies. An equal amount of serum was left unmixed and theoretically contained both egg white and egg yolk skin-sensitizing antibodies. One-tenth of one cubic centimeter of each serum was then injected intradermally in twelve sites on the outer upper arms of a nonallergic recipient, making a total of twenty-four sites, one-half injected with serum neutralized with egg white and one-half with serum alone. The sites were placed about two inches apart to allow ample space for testing antigens later.

The sites were carefully marked and the recipient, who had been on an egg-free diet for forty-eight hours, was required to return in forty-eight hours for testing with antigens of egg white, raw egg yolk, hard-boiled egg yolk, and commercial egg yolk.

All tests were done by the intradermal method. Testing strengths of egg white were 100 and 1,000 protein nitrogen units; if no reaction was elicited to either of these, a test was made with a 10,000 unit dilution of egg white. The three different egg yolk preparations were tested in strengths of 100, 1,000, and 4,000 protein nitrogen units. Tests were read five, ten, and fifteen minutes after injection of the antigen. Controls were performed for all tests. Only moderate or greater reactions (wheals of 0.5 cm. or larger) were considered as significant. All control tests were negative. All testing was done by two members of the group.

Of the sixteen patients studied, all of whom gave direct reactions to both egg white and egg yolk, only five were demonstrated to have transferable antibodies to egg white and three of these also had transferable antibodies to egg yolk. Two of the patients in whom the egg white antibody failed to transfer showed definite transferable egg yolk antibody.

Table I records the results obtained with the sera which showed passively transferable antibodies when they were tested with egg white and the three egg yolk antigens.

Our hard-boiled egg yolk preparation shows transferable sensitivity in three instances (sera II, III, and V) and commercial egg yolk in two of these cases (sera III and V). In each of these instances, however, the sensitivity was neutralized by the addition of egg white, indicating that the antigenicity was due to egg white rather than egg yolk.

In the case of raw yolk, six of seven sera gave a positive reaction to egg yolk. Of this number, four sera continued to give positive yolk reactions after

TABLE I. RESULTS OF PASSIVE TRANSFER USING EGG WHITE AND THREE EGG YOLK ANTIGENS

SERUM	EGG WHITE	COMMERCIAL YOLK	HARD-BOILED YOLK	RAW YOLK
I	+	−	−	+
I + EW*	−	−	−	+
II	+	−	+	+
II + EW	−	−	−	+
III	+	+	+	+
III + EW	+	−	−	+
IV	+	−	−	−
IV + EW	−	−	−	−
V	+	+	+	+
V + EW	−	−	−	−
VI	−	−	−	+
VI + EW	−	−	−	+
VII	−	−	−	+
VII + EW	−	−	−	+

*EW = Serum incubated twenty-four hours with egg white, 1,000 protein nitrogen units.

Seven sera with demonstrable egg white and/or egg yolk antibodies in which an attempt was made to neutralize the egg white antibodies.

Two sera (IV and V) show egg white antibodies only, and one (V) demonstrates some egg white antigen in all three yolks. Raw yolk was antigenic in six of the seven sera, while hard-boiled yolk and commercial yolk were antigenic in only three and two instances, respectively, all of which were neutralized by egg white.

complete neutralization of contained egg white. In serum V egg white neutralization eliminated all reaction, while in serum III insufficient egg white antigen was added to neutralize the egg white reaction.

This series of tests indicates that while neither a commercial preparation of egg yolk nor hard-boiled yolk is antigenic in itself, as demonstrated by passive transfer, fresh yolk is antigenic even when egg white antigen is eliminated. This is further confirmation of the fact that heating of foods aids in their allergenic denaturation. This also confirms the observations of Rubin and Major[8] that clinical sensitivity to egg yolk may exist in the absence of egg white sensitivity.

ANAPHYLACTIC STUDIES

All guinea pigs used in the anaphylactic studies weighed between 250 and 300 grams and had been fed a diet free of egg.

Four guinea pigs were given 12,000 protein nitrogen units of hard-boiled egg yolk intraperitoneally and twenty-five days later were given shocking doses intravenously, as follows:

GP 37. (1) 4,000 protein nitrogen units hard-boiled egg yolk. Slight reaction (scratching of the nose).
(2) 4,000 protein nitrogen units raw egg yolk. No reaction.

GP 38. (1) 0.2 c.c. fresh egg white 1:10. No reaction.

GP 39. (1) 4,000 protein nitrogen units hard-boiled egg yolk. Slight reaction (scratching of the nose).
(2) 4,000 protein nitrogen units of raw egg yolk. No reaction.

GP 40. (1) 0.2 c.c. fresh egg white 1:10. No reaction.
(2) 4,000 protein nitrogen units raw egg yolk. No reaction.

Four guinea pigs were given 12,000 protein nitrogen units of commercial egg yolk and twenty-five days later were given shocking doses intravenously, as follows:

GP 41. (1) 0.2 c.c. fresh egg white 1:10. No reaction.
(2) 4,000 protein nitrogen units commercial egg yolk. No reaction.
GP 43. Duplicated results obtained with GP 41.
GP 42. (1) 0.2 c.c. fresh egg white 1:10. No reaction.
(2) 4,000 protein nitrogen units fresh raw yolk. No reaction.
GP 44. Duplicated results obtained with GP 42.

Four guinea pigs were given 12,000 protein nitrogen units of raw egg yolk intraperitoneally and twenty-five days later were given intravenous doses, as follows:

GP 45. (1) 7,000 protein nitrogen units commercial egg yolk. No reaction.
(2) 4,000 protein nitrogen units raw egg yolk. Reactions occurred in thirty seconds and death in three minutes.
GP 46. (1) 0.1 c.c. fresh egg white 1:10. Reaction occurred in thirty seconds and death in three minutes.
GP 47. (1) 4,000 protein nitrogen units fresh raw yolk. Reaction occurred in thirty seconds and death in three minutes.
GP 48. (1) 7,000 protein nitrogen units commercial egg yolk. No reaction.
(2) 4,000 protein nitrogen units fresh raw yolk. Reaction occurred in thirty seconds and death in four minutes.

Interpretation of Results.—Slight, but significant, reactions occurred in two guinea pigs sensitized to hard-boiled egg yolk and shocked with the same material. No reactions of any kind were observed in those guinea pigs in which sensitization was attempted with commercial egg yolk. Anaphylactic death occurred only in those guinea pigs sensitized to raw egg yolk, but this method of testing did not indicate whether the anaphylactic reaction was due entirely to the raw egg yolk or to the egg white contaminant.

Because the results of this preliminary study of anaphylaxis produced by egg yolk were so consistent (that is, only egg white or raw egg yolk caused death in those pigs sensitized to raw egg yolk), and because there was no way to prove whether this shock was due to egg yolk or to adherent egg white, we decided that additional studies in anaphylaxis would not be helpful.

In order to obtain satisfactory proof that raw egg yolk is antigenic in the absence of egg white, and in order to be able to distinguish between the relative antigenicity of the various preparations of egg yolk, it became necessary to extend our experiments, using a more sensitive test.

ANTIGENIC STUDIES BY THE SCHULTZ-DALE TECHNIQUE

The Schultz-Dale test,[9] utilizing the uterine horns of the sensitized female guinea pig, is one of the more sensitive of immunologic techniques, and because the contraction elicited by the antigen on the sensitized muscle is initiated by the antigen-antibody reaction, the specificity of the reaction is the same as that of other serologic reactions.

Extracts of raw egg yolk, hard-boiled egg yolk, and commercial egg yolk were prepared in buffered saline and standardized according to the protein nitrogen content by the micro-Kjeldahl technique.

The raw egg yolk was especially prepared in an attempt to get an egg-white-free preparation as follows: An ordinary fresh egg was broken, the egg white was drained off, and the unbroken yolk was placed in a small flat dish. A continuous bath of normal saline solution was allowed to run over the yolk and drain off until all visible evidences of egg white were gone. Then the yolk sac was grasped with tissue forceps and a small opening was made with a pair of fine-pointed scissors. Through the opening thus made, a pipette was introduced into the yolk and the aspirated yolk was then transferred to sterile buffered saline for subsequent standardization.

All guinea pigs used were virgin females, weighing 250 to 300 grams, who had been fed a diet free of any egg. In the attempt at sensitization, all injections were given subcutaneously using 1 c.c. of an alum-precipitated extract containing 3,200 protein nitrogen units per cubic centimeter. Guinea pigs were injected with raw yolk, hard-boiled yolk, and commercial yolk.

PROCEDURE

The guinea pigs were killed twenty-five days after injection by a blow at the base of the skull. The uterine horns were removed immediately with as little trauma as possible and placed in Petri dishes containing Tyrode's solution. Unless the horns were to be used immediately, they were placed in a small reservoir in the Schultz-Dale muscle bath in which oxygen was constantly bubbled through Tyrode's solution. Each horn to be used was cut into small sections approximately 1.0 cm. in length. Preferably a section near the fimbriated end of the horn was used, since this area is usually more reactive.

The section of horn to be used is attached between the two hooks in the chamber of the muscle bath, the lower hook being fixed and the upper hook being attached to a thread connected to the muscle lever and writing arm. The chamber of the muscle bath is filled with Tyrode's solution maintained at 38° C. When the uterine horn is first suspended between the two hooks, it is in a state of clonic contraction, but after twenty to thirty minutes it gradually relaxes and the tracing of the writing arm on the kymograph, which is set at a speed of 3 mm. per minute, becomes a level, straight line. At this point the section of horn is ready to be used in testing.

The material to be tested is introduced into the muscle bath and in a matter of thirty to sixty seconds has circulated sufficiently to come in contact with the uterine horn. If the smooth muscle is sensitive to the material added

to the bath, the uterine horn will promptly undergo a strong contraction which produces a corresponding rise in the writing arm of the muscle lever. After the response is noted the Tyrode solution in the muscle bath is drained out and replaced by fresh solution, and the muscle is allowed to relax again before another material is added. The strength of the contractions varies with different guinea pigs, but it will be noted in our graphs that the maximum response to antigen obtained in each case was equal to that obtained with 0.05 mg. of histamine base.

RESULTS

Group I (Guinea Pigs Inoculated With Raw Egg Yolk).—Fig. 1 shows one horn of a guinea pig injected with 3,200 units of alum-precipitated egg yolk prepared as described above. The tracing shows a prompt contraction of the uterine horn when ovalbumin, 1,000 protein nitrogen units, is added. This

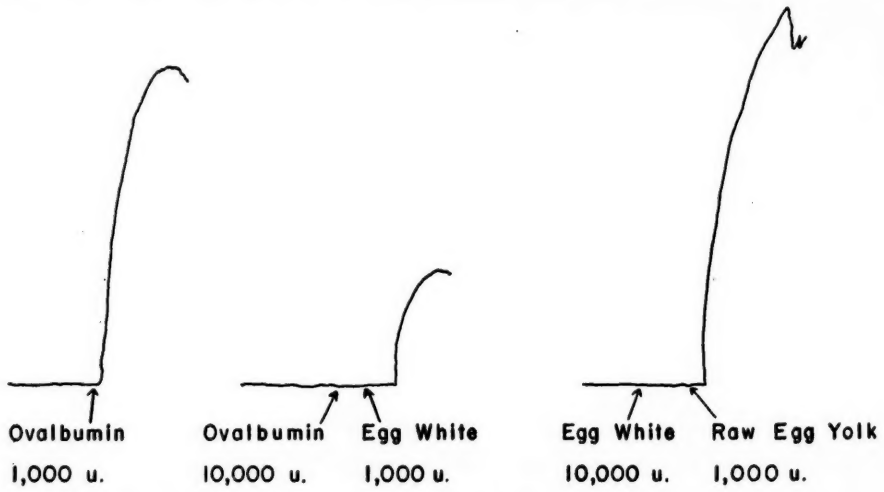

Fig. 1.—Demonstration of the antigenicity of egg yolk.

Upper: Guinea pig injected with 3,200 protein nitrogen units of alum-precipitated raw egg yolk prepared from washed and dissected egg yolks.

Lower: Kymogram of the reaction of one uterine horn of the sensitized guinea pig following stimulation in the Schultz-Dale muscle bath with the designated egg antigens. This indicates that the antigens of egg yolk and egg white are immunologically distinct, although egg white contains an antigen which is neither ovalbumin nor egg yolk.

indicates that even with the most meticulous care in the preparation of an egg-white-free yolk a small amount of egg white is present, although the ability to recognize it by this technique is probably due to the potentiation of its antigenicity by precipitation with alum. When ovalbumin no longer reacts, egg white as the antigen produces a moderate but prompt contraction of the uterine muscle, indicating the presence of an additional antigen present in egg white which is not ovalbumin. When egg white no longer produces a response, indicating that this additional antigen present in egg white is exhausted, then the addition of egg yolk causes a strong contraction of the uterine horn. Fig. 1 shows the presence in the uterine horn of a sensitivity to three distinct antigens—ovalbumin, egg yolk, and an additional antigen (not ovalbumin) evidently present in egg white.

Fig. 2 shows the results obtained in the second horn of the same guinea pig sensitized in the same manner with 3,200 units of alum-precipitated raw egg yolk.

Here there was a prompt contraction of the uterine horn, produced by the addition of the same egg yolk antigen to which the guinea pig was sensitized. After egg yolk failed to give a response on additional stimulation, neither ovalbumin nor whole egg white could be made to elicit a response. That the uterine horn was still active was proved by the addition of histamine, which gave a prompt contraction. This tracing, together with Fig. 1, indicates that in our egg yolk extract, meticulously prepared, ovalbumin, egg yolk, and the third antigen are present, since our egg yolk extract exhausted all antibodies to all three antigens. We were unable to confirm Coulson and Stevens'[7] work showing the presence of the common antigen in egg yolk, since we were unable to prepare an egg-white-free preparation of egg yolk.

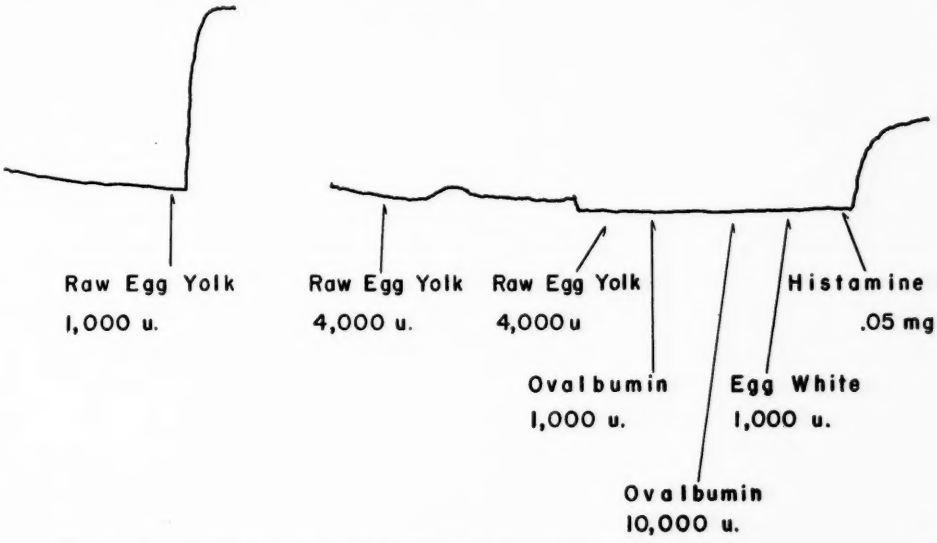

Fig. 2.—Demonstration of multiple antigens in egg yolk extract.
Upper: Second uterine horn of guinea pig shown in Fig. 1 sensitized to 3,200 protein nitrogen units of raw egg yolk.
Lower: Stimulation with homologous egg yolk extract exhausted all antibodies to ovalbumin and egg white as demonstrated to be present in Fig. 1.

Fig. 3 shows the results of a single experiment in a guinea pig sensitized to raw egg yolk prepared without any special care to remove or avoid egg white, such as might be done in the home.

Here the horn gave no reaction to commercial egg yolk and gave a prompt and vigorous response to fresh egg yolk. A second trial with fresh yolk gave a minimal response, while egg white produced a marked response comparable to the initial egg yolk response. After egg white failed to produce any reaction, histamine still produced a maximal response. From this, it is evident that guinea pigs sensitized to raw yolk as prepared in the home are readily and highly sensitized to both egg white and egg yolk, even without potentiation by alum precipitation. Again there was no reaction to commercial egg yolk.

Commercial Yolk .25 cc 4000 u/cc
Raw Yolk .1 cc 10,000 u/cc .15cc B.S.
Raw Yolk .1 cc 10,000 u/cc @ 15 cc B.S.
Egg White .1cc 10,000 u/cc
Egg White .1 cc 10,000 u/cc
Egg White .1cc 10,000 u/cc
Histamine .1 cc .05 mgs/cc

Fig. 3.—Demonstration of antigenicity of raw egg yolk as prepared at home.

Upper: Guinea pig injected with 9,000 protein nitrogen units of egg yolk prepared by washing only.

Lower: Kymogram of the reaction of the uterine horn of the sensitized guinea pig following stimulation in the Schultz-Dale muscle bath with the indicated egg antigens.

Commercial yolk causes no contraction, while the presence of egg yolk and egg white antibodies in the muscle is demonstrated by prompt reactions.

Group II (Guinea Pigs Inoculated With Hard-Boiled Egg Yolk).—Fig. 4 represents results from an experiment with a single horn of a guinea pig sensitized to 3,200 protein nitrogen units of alum-precipitated hard-boiled egg yolk extract.

This horn developed prompt contraction when exposed to egg white but failed to respond to a second stimulation with egg white. It also failed to respond to contact with hard-boiled yolk or raw yolk.

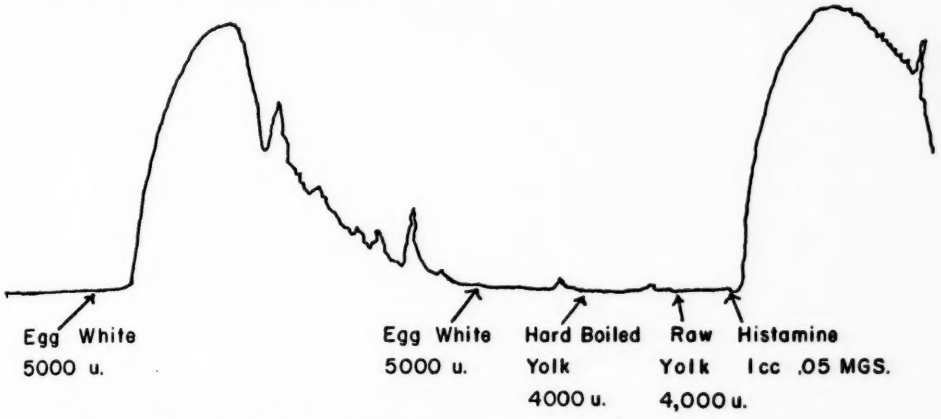

Fig. 4.—Demonstration of egg white sensitization by hard-boiled yolk.

Upper: Guinea pig injected with 3,200 protein nitrogen units of alum-precipitated hard-boiled egg yolk extract.

Lower: Kymogram of the reaction of the uterine horn of a sensitized guinea pig following stimulation in the Schultz-Dale muscle bath with the designated antigens.

There is a prompt response to egg white, but no response to egg yolk, showing the absence of sensitization to hard-boiled egg yolk.

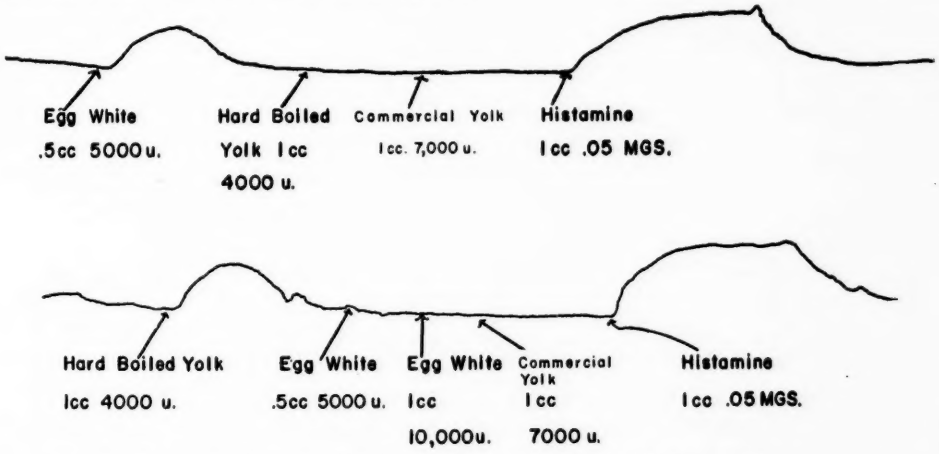

Fig. 5.—Demonstration of presence of egg white in hard-boiled egg yolk.

Upper: Guinea pig injected with 3,200 protein nitrogen units of alum-precipitated hard-boiled egg extract.

Lower: Kymograms of reactions of the uterine horns of the sensitized guinea pig following stimulation in the Schultz-Dale muscle bath of the designated antigens.

Upper tracing confirms tracing in Fig. 4 and, in addition, shows a lack of response to commercial yolk.

Lower tracing elicits a response to hard-boiled yolk extract, but fails to respond to egg white or commercial yolk, indicating that the guinea pig injected with hard-boiled egg yolk is sensitized to egg white but not to egg yolk.

Fig. 5 shows another guinea pig sensitized to hard-boiled yolk in the same manner as the preceding one. Here the upper tracing confirms the results of the tracing in Fig. 4, with the additional fact that commercial yolk also failed to produce any response. The lower tracing shows that hard-boiled yolk gave a response almost as great as egg white and that after the response to hard-boiled yolk, egg white and commercial yolk failed to give any contraction although the horn still responded to histamine. These results are taken to mean

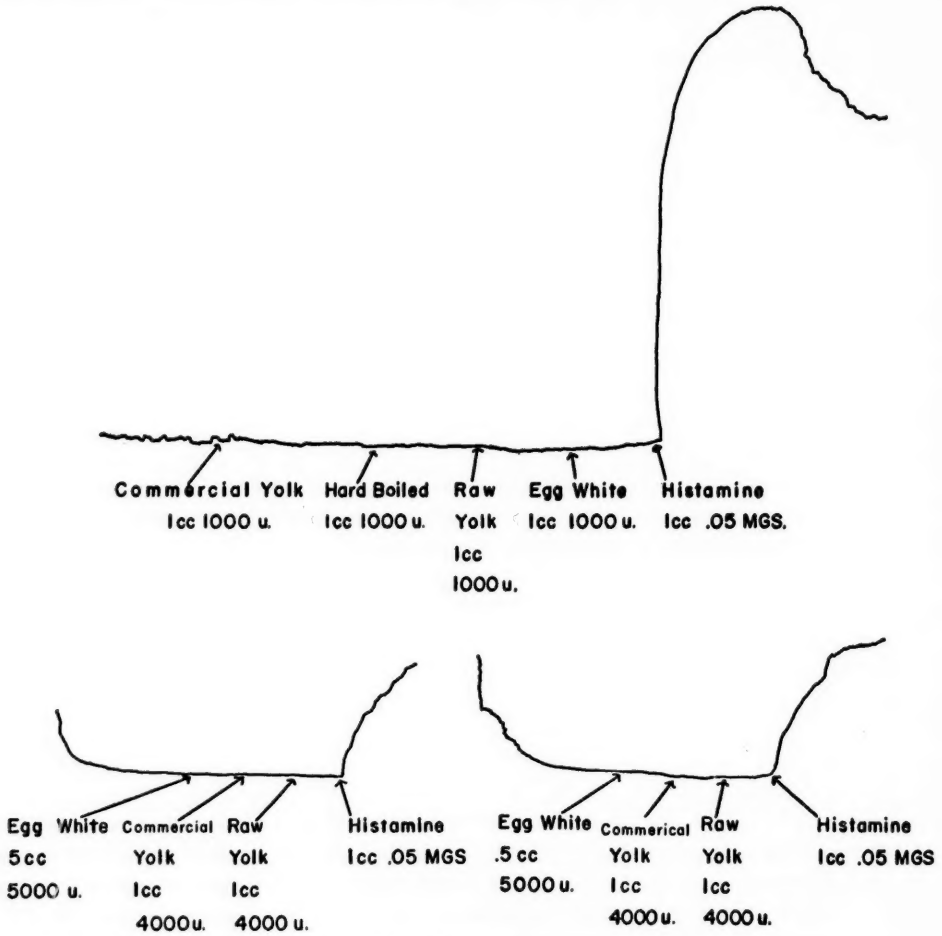

Fig. 6.—Demonstration of nonantigenicity of commercial egg yolk.

Upper: Guinea pigs injected with 3,200 protein nitrogen units of commercial egg yolk extract.

Lower: Kymograms of reactions of the uterine horns of the guinea pigs following stimulation in the Schultz-Dale muscle bath of the designated antigens.

These horns failed to respond to all egg preparations, but responded promptly to histamine.

that guinea pigs injected with hard-boiled yolk have promptly become sensitized to egg white but have failed to show any sensitization to either hard-boiled yolk or commercial yolk. In contrast, as shown by preceding tracings,

sensitization to raw egg yolk was shown to be produced readily. The possibility of the presence of the common antigen cannot be excluded from these two tracings.

Group III (Guinea Pigs Inoculated With Commercial Egg Yolk).—Fig. 6 represents three tracings of horns from three guinea pigs injected with 3,200 units of commercial yolk in the alum-precipitated form. In no instance did the uterine horn react to anything but histamine, in spite of potentiation of antigenicity by alum precipitation, indicating that the smooth muscle has not been sensitized to either egg yolk or egg white as a result of contact with commercially prepared egg yolk.

CONCLUSIONS

From the foregoing data, the following conclusions appear to be justified:

1. The Schultz-Dale technique appears to be one of the most satisfactory methods of studying the antigenicity of a substance where more than one antigen is involved.

2. Raw egg yolk is capable of antigenic stimulation, and this can best be demonstrated clearly and satisfactorily by both the passive transfer and Schultz-Dale tests.

3. The sensitivity produced by raw egg yolk is separate and distinct from the sensitivity produced by egg white, although there is evidence of a separate antigen common to both.

4. Sensitivity to egg yolk could be demonstrated to occur only with raw egg yolk and not with hard-boiled yolk or the commercial preparation, by either passive transfer or the muscle bath technique.

5. Hard-boiled yolk and commercial yolk showed antigenically significant amounts of egg white antigen in passive transfer studies, although they were appreciably less than those present in raw yolk.

6. By the Schultz-Dale technique and anaphylactic studies on guinea pigs, the commercial yolk used in this study appeared to be nonantigenic with respect to egg yolk, egg white, or a common antigen. This is further confirmation of the fact that heating of foods aids in their allergenic denaturation.

7. Because it is well known that the results of animal experimentation, no matter how carefully performed, cannot always be applied with impunity to the human being, it is strongly advised that utmost caution be exercised in the feeding of any preparation of egg yolk or egg white to an infant or child known or suspected of being sensitive to egg.

We wish to express our appreciation to E. J. Coulson, Ph.D., United States Department of Agriculture, for his assistance and his many helpful suggestions.

REFERENCES

1. Warner, Robert C.: Egg Proteins, in: Neurath, H., and Bailey, K. (editors): The Proteins, New York, 1954, Academic Press Inc., Vol. II, Part A, pp. 435-485.
2. Marshall, Margaret E., and Deutsch, H. F.: Distribution of Egg White Proteins in Chicken Blood Serum and Egg Yolk, J. Biol. Chem. **189**: 1-9, 1951.
3. Onoe, Tamenobu: Biochemical Studies on Carbohydrates. XVI. Vitello-mucoid, J. Biochem. (Japan) **24**: 1-8, 1936.

4. Fränkel, S., and Jellinek, C.: Ueber die sogenannte Kohlehydratgruppe im Eiweiss (Darstellung der Glucosamino-Mannose), Biochem. Ztschr. **185:** 392-399, 1927.
5. Hektoen, Ludvig, and Cole, Arthur Gideon: The Proteins of Egg White: The Proteins in Egg White and Their Relationship to the Blood Proteins of the Domestic Fowl as Determined by the Precipitin Reaction, J. Infect. Dis. **42:** 1-24, 1928.
6. Lewis, Julian H., and Wells, H. Gideon: The Immunologic Behavior of Mucoids, J. Infect. Dis. **40:** 316-325, 1927.
7. Coulson, E. J., and Stevens, Henry: Allergenic and Anaphylactogenic Properties of Vaccines Prepared From Embryonic Tissues of Developing Chicks. II. Anaphylactogenic Properties of Typhus-Fever Vaccines and Equine Encephalomyelitic Vaccines, J. Immunol. **53:** 321-342, 1946.
8. Rubin, Simon S., and Major, M. C.: An Allergic Reaction Following Typhus-Fever Vaccine and Yellow-Fever Vaccine Due to Egg Yolk Sensitivity, J. ALLERGY **17:** 21-23, 1946.
9. Coulson, E. Jack: The Schultz-Dale Technique, J. ALLERGY **24:** 458-473, 1953.

REPEATED ARTHUS DESENSITIZATION

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PREVIOUS reports[1, 2] have shown that Arthus desensitization can be maintained by large daily doses of antigen and that *prolonged* desensitization leads to a temporary state of immunologic exhaustion. A number of unanswered questions were raised.

This experiment was designed to determine: (1) what doses of antigen would be required; (2) how often sensitization would return after repeated desensitization; and (3) when and whether lasting desensitization and immunologic exhaustion would follow repeated *short* periods of desensitization.

METHOD

Two rabbits were sensitized by repeated intradermal injections of 0.1 c.c. of 1:5 egg white solution into four separate sites every five days until, on the thirty-seventh day, they developed 4+ Arthus skin reactions[1, 2] and ring precipitin titers of 10^6 or stronger. A 1:5 egg white solution was used intravenously for desensitization and 1:20 was given intradermally for the skin tests. Reasons for using egg white and the ring precipitin test have been discussed previously.[1, 2]

The rabbits were then alternately desensitized and allowed to recover. The sizes and frequency of the antigen doses, the duration of each desensitization and recovery period, and the averaged titers of the skin reactions and of the precipitin tests in the two rabbits are shown in Fig. 1. A total of seventy-five skin tests and sixty precipitin tests were done on each animal during the 233 days of the experiment. For the sake of simplicity, only those titers which show the minimum desensitization, the maximum averaged recovery titers of the skin reactions and precipitins, and the need for larger desensitizing doses (Stages IV and VIII) are shown in Fig. 1. To save space, the duration of each stage is indicated in days by a number in the figure and this number is not related to the width of the columns representing the stages.

The duration of the desensitizing and recovery periods was determined arbitrarily. In general the sizes of the desensitizing doses of antigen were increased daily until skin and precipitin titers were minimal. Antigen injections were then discontinued until there was no further increase in successive skin and precipitin titers, which were done every two or three days during the

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recovery periods. Blood was drawn for precipitins and antigen was injected to elicit the skin reactions about two hours after each desensitizing dose of antigen. Skin reactions were read about twenty-four hours later. They were recorded as of the date they were done, and not the date they were read.

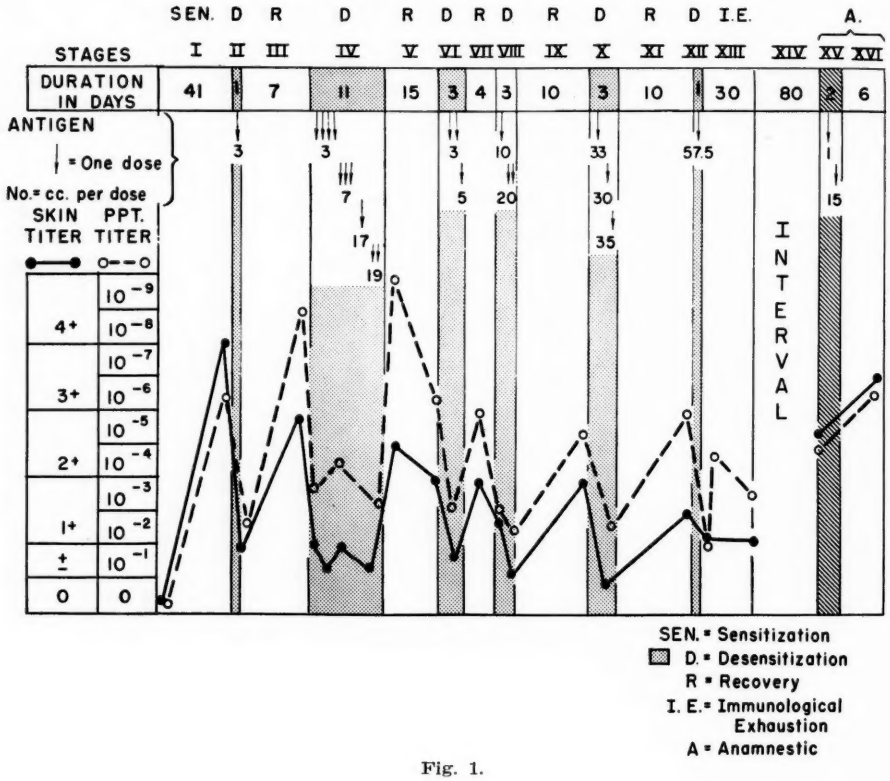
SEN. D R D R D R D R D R D I.E. A.
STAGES I II III IV V VI VII VIII IX X XI XII XIII XIV XV XVI
DURATION IN DAYS 41 1 7 11 15 3 4 3 10 3 10 1 30 80 2 6
ANTIGEN
↓ = One dose
No.= cc. per dose
SKIN TITER
PPT. TITER
4+ 10⁻⁹ 10⁻⁸
3+ 10⁻⁷ 10⁻⁶
2+ 10⁻⁵ 10⁻⁴
1+ 10⁻³ 10⁻²
± 10⁻¹
0 0
3
3 7 17 19
3 5
10 20
33 30 35
57.5
1 15
INTERVAL
SEN. = Sensitization
D. = Desensitization
R = Recovery
I. E. = Immunological Exhaustion
A = Anamnestic

Fig. 1.

RESULTS

1. Marked reductions of the skin reactions and precipitin titers followed the first desensitizing dose of 3.0 c.c. of 1:5 egg white solution (Stage II). This dose was given on three consecutive days in Stage IV with similar results, but both the skin reactions and precipitin titers rose slightly on the fourth day. Progressively larger daily doses were given (5, 7, 19 c.c.) before this rise was reversed. Desensitization was marked in Stage VI, after a total of 11 c.c. of antigen in three days. Thereafter, in Stages VIII, X, and XII, much larger doses were required to obtain the comparable reductions in skin reactions and precipitin titers.

2. Precipitin titers rose above predesensitization levels in the first two recovery periods (Stages III and V). Recovery peaks were progressively lower thereafter (except in Stage XI).

3. The peak of intensity of the skin reactions decreased progressively in each recovery period until Stage XIII, during which skin reactivity remained

at the desensitization level. This is interpreted as lasting desensitization coincident with immunological exhaustion.

4. Maximum rises in skin and precipitin titers during the recovery periods were noted within six days or not at all, with the exception of some peaks on the eleventh day in Stage V.

5. Individual skin reactions became negative four times and were equivocal fourteen times during the desensitization periods. These are not shown in the figure which shows the averages of two skin reactions.

6. Precipitin titers were below 10^3 only three times during the desensitization period.

7. The behavior of the two animals was remarkably similar. On eight occasions the skin reactions were 1+ in one animal and 2+ in the other, and once they were negative in one animal and 1+ in the other. All other readings were the same. The paired precipitin titers were identical or differed by one dilution with the exception of four occasions when they differed by two dilutions and three others which differed by three dilutions. The parallelism between skin reactions and precipitin titers was impressive.

8. During the interval Stage XIV, which lasted eighty days, injections of 16 c.c. of egg white on the tenth and thirty-fifth days were followed by negligible changes in the skin and precipitin titers. Shortly before Stage XV the skin reactions and precipitins rose to the titers shown. Six days after the anamnestic dose of 15.0 c.c. of the antigen, the skin and precipitin titers returned to predesensitization levels. The experiment ended at that point.

DISCUSSION

Preliminary sensitization with small intradermal doses was done in the hope that desensitization could be maintained with smaller desensitizing doses. This was not the case, however. After the first effective small desensitizing dose of 3.0 c.c. of antigen, progressively larger doses were required to bring about comparable reductions of skin reactivity and precipitin titers. For example, in Stage VIII, 50.0 c.c. was required, in three days, to lower the titers to the same levels to which they had fallen after the 3.0 c.c. dose of antigen in Stage II. And in Stage XII, 57.5 c.c. in one day caused less reduction of the skin reaction than had 3.0 c.c. in Stage II.

Skin reactivity was minor after the fifth desensitization. This state of apparent immunologic exhaustion or lasting desensitization was observed on the seventieth day after the intial desensitizing dose of antigen. Recovery of skin reactivity and precipitin titers to predesensitization levels was complete in 120 days, in this experiment.

A similar state was observed on the eightieth day in the previous experiment.[2] The only common factors in the two experiments, which led to immunologic exhaustion and prolonged desensitization, were the large doses of antigen and repeated desensitization. There was no correlation in the two experiments between the total doses of antigen, the number and duration of desensitizing periods, the number of days antigen was given, the doses and

routes of sensitizing antigen, or of the size of the desensitizing doses. Both animals remained healthy, in contrast to those in the preliminary experiment.[1]

The roles of minor antigens and antibodies, the so-called "negative phase" of antibody depression, antibody rebound, the relationship of immunologic exhaustion to immunologic paralysis and immunologic unresponsiveness, the duration of desensitization, persistence of the low precipitin and skin reaction titers, and other problems were discussed in the previous reports.[1, 2]

The fate of the large doses of antigen, estimates of the amount of antibody produced, proportions of antibody to nonantibody globulins, and factors which determine the time of appearance and disappearance of the immunologic exhaustion and prolonged desensitization should be determined later.

Further studies of Arthus desensitization and immunologic exhaustion and related problems will be deferred until a pure antigen which does not give rise to antibodies to minor antigenic constituents is available. Crystalline egg albumin, purified bovine albumin, and gamma globulin are not sufficiently free of minor antigens to be used profitably in experiments which require such large doses.

SUMMARY

1. Repeated Arthus desensitization required progressively larger doses of antigen.

2. When periods of desensitization and recovery were alternated, the maximum intensity of the skin reactions decreased progressively with each recovery period. A similar decrease in precipitin titers was observed after the second recovery period.

3. Prolonged desensitization coincident with immunologic exhaustion was observed after the sixth period of desensitization, seventy days after the first desensitizing dose.

4. Predesensitization skin reactivity and precipitin titers returned in response to anamnestic injections of antigen, 120 days after immunologic exhaustion.

5. A pure antigen which will not elicit minor antibodies is necessary for the solution of a number of problems raised by this and previous experiments.

REFERENCES

1. Swineford, O., Jr., Agee, Betty A., Carpenter, J. T., Jr., Fitch, E., and Tull, L.: Maintenance of Arthus Desensitization, J. ALLERGY **27**: 143, 1956.
2. Swineford, O., Jr., Hoch-Ligeti, C., Irvine, K., Fitch, E., and Quelch, S.: Arthus Desensitization; Studies of the Antibodies, Skin Reactivity, and Serum Proteins in Lasting Desensitization, J. ALLERGY **28**: 142, 1957.

American Academy of Allergy

REPORT OF THE RESEARCH COUNCIL
1956-1957

THE period covered by this report has been a period of consolidation for the Research Council. In 1955, several new subcommittees were activated, for instance the Subcommittees on Insects and on Weather; none was activated in 1956. The activities of the existing committee, on the other hand, have expanded, and blueprints for further expansion have been prepared.

I. COMMITTEE ON AIR-BORNE ALLERGENS

The structure of the Committee on Air-borne Allergens remained the same.

1. Subcommittee on Pollen and Molds

Herbarium.—Eight herbarium specimens were contributed to the collection. The total number of species of plants represented in the total pollen collection approximates 260. The number of species of plants represented in the total herbarium collection approximates 300. Pollen specimen slides sent in response to thirteen requests total 294.

Statistical Report.—

A. *Pollen:* More than sixty allergists, botanists, and public workers are turning in reports of about 100 pollen surveys for this past year. New work has been started in a number of places, including several foreign countries, notably Venezuela and the Philippines. The secretary spent four days helping set up an intensive survey in Salt Lake City and vicinity.

For the coming year, we plan to continue such special projects as the Sunland-Tuhunga study, the campaign to keep ragweed out of Western Oregon, and a three- or four-year study of pollen counts taken four miles out on Lake Michigan as compared with counts made in Chicago. A survey will be made at Lake Tahoe, California.

B. *Molds:* During 1956, mold counts were received from approximately forty different cities in the United States and Canada. Graphs for some of these cities, particularly those on the Pacific Coast, were part of the mold exhibit in Los Angeles.

Many sections of the country as yet have had no mold surveys. Attempts will be made to stimulate some interest in this field to men practicing in those sections.

Exhibits.—A scientific exhibit—"Air-borne Mold Spores in Seasonal Allergy"—was shown at the American Medical Association meetings in Chicago

in June, 1956, by Mr. Oren C. Durham and Dr. David Merksamer. The exhibit received an "honorable mention." The exhibit was subsequently shown at the clinical meetings of the A.M.A. in Seattle in November, 1956, and brought to Los Angeles for the meetings of the American Academy of Allergy. At each of the exhibits, a considerable number of the Committee's pamphlets and reports were distributed.

Officers, Members, and Collaborators.—Oren C. Durham, now at Box 33, Angwin, California (Secretary for Pollen), David Merksamer (Secretary for Molds), Clarence Bernstein, Jose L. Cortes, A. O. Dahl (Technical Consultant), Sidney Friedlaender, Frank T. Joyce, Eloise W. Kailin, Dean A. Moffat, Frank Perlman, Roland T. Rooks, Bram Rose, Grace M. Talbott, Abraham M. Targow, Albert M. Tocker, Lester C. Todd, Martyn A. Vickers, Ralph F. Voigt (Curator of the Herbarium), Charles H. A. Walton, and Matthew Walzer.

About 100 allergists, public health officers, and botanists are carrying out local surveys throughout North America.

2. Subcommittee on Dust

The Subcommittee on Dust has been inactive during 1956 and 1957. It is apparent that the dust problem has reached a stage where it requires efforts of individual investigators rather than the efforts of the Research Council. Work on the chemical and immunologic identification of the active components of house dust extract is progressing in several laboratories. Once the active fractions have been isolated, the facilities of the Research Council will be invaluable for large-scale testing.

Francis C. Lowell, M.D., Secretary
65 East Newton St.
Boston, Massachusetts.

3. Subcommittee on Insects

A. *Activities.*—Insect allergens have been supplied on request to many physicians. Many new insect allergens have been prepared and will be studied in the future. A more comprehensive bibliography on insect allergy is being prepared and abstracts of interesting articles are being compiled.

B. *Projects.*—We hope to gain better reports on the effects of insect allergens which were supplied to the various physicians. New insect allergens will be distributed among members of the group. Further studies will be carried on concerning techniques for preparing and standardizing such allergens. Further assistance will be sought of others in compiling abstracts of literature on insect allergy. An attempt will be made to compile an informal report of comments and inquiries on cases and problems in insect allergy.

Members.—Richard L. Etter, Houston, Texas; Alan R. Feinberg, Chicago, Illinois; Frank Rawlings, Toledo, Ohio; Henry Stevens, Washington, D. C.;

Robert A. Stier, Spokane, Washington; Boen Swinney, San Antonio, Texas; Martyn A. Vickers, Bangor, Maine.

Frank Perlman, M.D., Secretary
406 Medical-Dental Bldg.
Portland 5, Oregon.

4. Subcommittee on Weather

The Subcommittee on Weather was activated at the 1956 meeting of the American Academy of Allergy.

The Subcommittee designed a form, based on the suggestion of members, which allowed uniform reporting of clinical information and a convenient transfer of data to IBM cards.

Clinical data included day, date, and hour of the onset of symptoms of bronchial asthma. If a sufficient percentage of patients in a given area developed bronchial asthma at the same time, attempts were made to identify the weather changes which took place during the period of aggravation. If such a change recurred subsequently, arrangements were made to notify the appropriate committee member and to alert him to determine whether his patients had a recurrence of the difficulties.

The area consultant for the United States Weather Bureau in each locality has been in touch with the local Subcommittee member and is prepared to furnish weather data when requested. In addition, information is obtainable from the collection center of the Weather Bureau in Asheville, North Carolina.

The secretary met with Dr. Gerald Barger who had been appointed to coordinate the United States Weather Bureau's activities with the Subcommittee. Dr. Barger agreed to meet with the secretary again when more clinical data became available.

Of the original members of the Subcommittee who were appointed, eight members have responded by returning cards on patients. Some of the others may be collecting cards, but the secretary at this time has no knowledge of the number of patients or the amount of data being collected.

The secretary arranged with the IBM Service Center in Denver, Colorado, to punch-code the data which have been sent in to date. Reports from two cities were so processed. A majority of the patients developed symptoms on a specific day and at a specific hour in each instance. The weather data for these times are being studied currently.

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5. Subcommittee on Air Processing

In its early years, the Air Processing Committee, a subcommittee of the Committee on Air-borne Allergens, cooperated with the United States Bureau of Standards in tests which compared the capacity of window filters to remove ragweed pollen supplied by the Research Council. Shortly afterward, the

Bureau of Standards had to withdraw from this joint project. The Subcommittee then enlisted the aid of the Farr Manufacturing Company of Los Angeles, California. The air filter testing equipment which has been developed in the laboratories of the Farr Manufacturing Company permits air to be forced at any desired rate through any type of filter. Pressure drop across the filter can be measured and particulate matter can be fed into the upstream side at a measured rate. It is possible to measure efficiency by either of two methods:

1. Gravimetric, that is, through weighing a filter placed at the end of the wind tunnel before and after each run.
2. By millepore sampling of the air upstream and downstream from the filter.

The Farr Company has been most cooperative, and stands ready to run tests on new filter devices under the supervision of this committee. Reports of such tests are submitted to the committee chairman and, with his observations, are transmitted to the chairman of the Committee on Aero-Allergens and to the secretary of the Research Council who, in turn, make the final report to the company which has requested the tests. The cost of this service to the manufacturer is minimal and covers only the actual cost of the service.

After these several years of experience, it might be appropriate to reiterate the general principles of our tests so that the individual allergist will be in a position to evaluate an air-filtering device by applying our general standards to his specific problem.

Allergists are concerned with:

1. The outside air, and the following possible offenders in it: (a) pollens, (b) mold spores, (c) organic dusts, and (d) gases and aerosols.

2. The inside of the home, particularly with house dust in its broadest sense. Ideally, the allergic person who is potentially sensitive to many of these factors should live in a house in which he is protected from all of them. With the technical advances made in the last ten or fifteen years, he can have such a home if he can afford the necessary investment. In order to achieve this degree of protection, a system can be designed which requires:

1. Forced-air heating and air-conditioning
   - (a) blower
   - (b) ducts
   - (c) furnace
   - (d) air-conditioning equipment
2. Impingement filters
3. Electrostatic precipitators
4. Activated charcoal filters.

Such a system demands that a large proportion of the air be recirculated; that windows and outside doors remain closed, except when it is necessary for the

doors to be opened for ingress or egress; and that the sources of house dust within the house be kept at a minimum by the use of foam rubber in mattresses and upholstery.

One might immediately ask why three filtering devices are necessary; this is, indeed, a valid question. Activated charcoal filtration is the only practical method of removing noxious gases and aerosols. In general, this type of filter may be eliminated in most communities since there is little evidence that smog or noxious gases in common concentrations are implicated in the etiology of hay fever or bronchial asthma.

Impingement filters with which we are most familiar are all built on the same principle. They consist of a maze of fibers which are coated with a sticky substance. Gases which have no particulate weight pass through the maze, but particulate matter, if it is large enough, will strike the coated fiber and, because of its inertia, will be held in the filter simply by adhering to its coating. Two factors determine whether or not an individual particle will be caught by the filter:

1. Size. The larger particle is more likely to be caught.
2. The weight or specific gravity (the greater the specific gravity, the greater the inertia, that is, the likelihood of being caught). It can be shown that a particle of sand which has a small diameter but a high specific gravity (for instance, SiO_2 which has a specific gravity of 2.2) will be caught more readily than a particle of organic material which has the same diameter but a specific gravity of 1.1.

The diameter of pollen grains varies from 10 to 200 micra. Ragweed pollen grains are 15 to 20 micra in diameter. Allergenically important mold spores vary in diameter from 1 to 40 micra. House dust particles may be of almost any size, but we can be reasonably certain that the smaller ones—say, from 0.1 micron to 10 micra—are probably the most important from the point of view of the allergenic patient, as the particles in this size range settle very slowly and therefore tend to remain in the air which the patient breathes for relatively long periods of time.

We know from various tests over a period of years that the ordinary glass wool oil-coated impingement filter will remove approximately 75 per cent of pollen from air passing through it; its efficiency in removing a standard inorganic dust with particles of a size up to 5 micra is less than 25 per cent. One may deduce from these figures that its efficiency with relation to fine house dust or small mold spores is too low to be useful. One impingement filter, unfortunately made only for commercial installations, is approximately 98 per cent efficient in its ability to remove ragweed pollen, but even this filter is able to remove only about 10 to 20 per cent of particles of a standard inorganic dust 5 micra or less. In other words, even a good impingement filter would allow allergenic material to pass through into the air which the patient breathes.

The electrostatic precipitator (not to be confused with certain polystyrene, polyethylene, and other viscose filters which will be discussed later in this report) consists of fine wires carrying a positive charge of 6,000 to 15,000 volts and a series of plates carrying a negative charge of 6,000 to 15,000 volts. The air to be filtered passes by the wires which impart a positive charge to all the particulate matter contained in this air. When these particles enter the field of the negatively charged plates, the positively charged particles are powerfully attracted to the plates and are thereby removed from the air. A variation of design uses a coarse screen instead of plates, but the basic principle is the same. The Subcommittee has examined a filter pad and precipitator combination in which a much lower voltage was used, but found this to be inefficient. It will be noted that the larger and heavier particulate matter passes through the electrostatic precipitator but will be removed by impingement filters. The electrostatic precipator, however, is extremely efficient in the removal of small particles. Our tests have shown it to be almost 100 per cent efficient for ragweed pollen and better than 80 per cent efficient for particles 5 micra or less in diameter. It should be evident from the foregoing that these two filters complement each other and, when used in conjunction with each other, remove practically all particles from the air. Even the electrostatic filter is only 80 per cent efficient for the very small particles, but when one takes into account that between 75 and 80 per cent of the air is recirculated, it becomes evident that the house air will be free of dust after the system has been in operation for a few hours if mattresses or overstuffed furniture containing cotton linters are eliminated as a source of recontamination.

One filter exists which can take the place of both the impingement filter and the electrostatic precipitator, but this filter (the Cambridge Absolute Filter) requires a high-pressure blower to force the necessary amount of air through it. The initial cost of this blower would be several times that of an ordinary low-pressure blower, and the cost of operation would be considerably higher. Practically all the heating and air-conditioning systems which are ordinarily installed include the use of an impingement type of filter, but few use the electrostatic precipitator. The physician who recommends the latter should understand its limitations.

For the patient who is sensitive to pollen, mold, and house dust, a "complete" system can assure him of a practically allergen-free home, but the cost is high. The electrostatic filter alone will add approximately $700.00 to the cost of the forced-air heating system if this system delivers between 1,500 and 2,400 cubic feet of air per minute; there is, however, no satisfactory substitute which is less expensive.

Patients who are sensitive to house dust only can eliminate dust-producing materials in the home and derive some help from a small room-model electrostatic precipitator which can be operated in any desired room. Such units are available and will recirculate the room air at the rate of 100 to 200 cubic feet per minute. The approximate cost of these portable units is $200.00.

Patients who are sensitive to pollen only will derive significant protection from window filters. The best of these removes approximately 95 per cent of the pollen from the air and use a paper type of filter. Filters which use glass wool are less efficient, and permit nearly 25 per cent of the pollen in the air to escape through them. It can be readily seen that within a short time the air within the room supplied through such a filter must become heavily contaminated with pollen. In the past three or four years a new type of filter has appeared on the market—the so-called electrostatic filter made of viscose fibers. As air passes through these filters, electrostatic charges are set up, and these charges tend to make particulate matter adhere to the fibers. These filters are fairly efficient when they are new, but as they accumulate dust they develop the unfortunate tendency to "unload." This simply means that, when dirty, they become less efficient, a characteristic which is not shared by the impingement filters; these latter two merely clog up with dust and increase resistance to the current of air.

At the meeting of the American Academy of Allergy in New York in 1955, Dr. Walter S. Burrage* reported on a combination of air-conditioner and electrostatic precipitator which had been produced experimentally by the Raytheon Company. The unit was highly efficient in filtering pollen and provided adequate cooling which adds to comfort during most of the grass and much of the ragweed seasons. It seems unfortunate that the company decided not to manufacture this satisfactory unit. Somewhat later a similar combination of a (different) electrostatic air filter and air-conditioner was tested by this Committee, but it failed to remove an adequate amount of pollen from the air. At a recent meeting of the American Academy of Allergy, a new combination of air-conditioner and electrostatic precipitator was demonstrated, but the Subcommittee has not yet had an opportunity to test this unit.

The reasons for the Raytheon Company's unwillingness to go ahead with serial production of their effective unit are interesting. The company felt that: (1) the additional cost would bring the price of the unit too far above the price of competitive air-conditioners; (2) the added depth (approximately 10 inches) would make the unit too bulky for ordinary windows; and (3) the pollen-sensitive person could purchase a standard window air-conditioning unit plus a portable electrostatic precipitator for a more attractive price.

The Subcommittee believes that the window combination of electrostatic precipitator and air-conditioner is preferable, since it prevents pollen and dust from entering the room, but agrees that the combination of window air-conditioning unit and portable electrostatic precipitator offers the best compromise until combined window units become available.

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*Novy, H. S., Irwin, J. W., Welts, J., and Burrage, W. S.: Removal of Ragweed Pollen From Environmental Atmosphere of Patients, J. ALLERGY 27: 398, 1956.

II. COMMITTEE ON CONTACTANTS

In 1956, an inquiry about the need for a list of sensitizing contactant substances (to be used as a guide for patch tests) was sent to the members of the Committee. Of the replies received, most expressed the opinion that the choice of materials was subject to individual requirements. Several extensive lists of such materials and of the proper concentrations for their use are available. One or two replies expressed the need for a list of minimal test substances. Informal discussions of the subject have been scheduled, and a final summary of the survey should become available during the coming year. As before, the Committee on Contactants has functioned as an advisory committee. Plans for reactivation of its investigative program are under consideration.

Officers, Members, and Collaborators.—Dr. W. C. Spain, one of the members of the Committee on Contactants, died in 1956. Drs. John L. Fromer of Boston, Massachusetts, and Albert M. Kleigman of Philadelphia, Pennsylvania, have become members of the Subcommittee on Testing Procedures—Man. Otherwise, the roster of members and advisers of the Committee has remained unchanged since the last report.

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III. COMMITTEE ON DRUGS*

The work of the Committee on Drugs can be divided into four general categories:

1. Evaluation of new drugs, or old preparations with new applications.

2. Advice to the Food and Drug Administration concerning: (a) new drug applications, (b) request for over-the-counter dispensing of older drugs, and (c) allergic reactions to drugs in general use.

3. Answering of questions from the medical profession, pharmaceutical manufacturers, and the public concerning drugs applicable to the treatment of allergic disease. Some of these inquiries are referred from Academy headquarters, and others from Members and Fellows of the American Academy of Allergy.

4. Investigation of unsubstantiated claims for the drug treatment of allergy, appearing in medical journals or in the public press. These usually involve unknown preparations, or drugs which have no previous documented background of successful use in allergic disease.

In the first, and major, category three (out of five) accepted projects were completed in 1956. Several drugs were unacceptable because they did not meet the requirements specified by the Committee in its report to the Research Council for 1955-1956 (J. ALLERGY **27**: 273, 1956). Tests were carried

*This brief summary will be supplemented by a separate report of the Committee on Drugs which will enlarge upon some of the activities mentioned here.

out and completed on: (1) a bronchodilator, "JB-251" (Lakeside Laboratories), an N-substituted arterenol; (2) an antihistaminic-resin complex (R. J. Strasenburgh Company); and (3) prednisone and prednisolone in nonseasonal allergies (Schering Corporation).

Tests still in progress include: (1) zinc-ACTH (Organon, Inc.) and (2) gamma globulin in infectious bronchial asthma (The American National Red Cross).

The study of the effectiveness of gamma globulin in bronchial asthma associated with bacterial infection is a long-term project expected to cover a period of two years.

Advice to the Food and Drug Administration in all three categories outlined above was rendered during the year. Opinions given reflected those of the majority of the Committee as well as selected Members and Fellows of the Academy outside of the Committee, who were known to have a special knowledge or interest in the particular problem at hand. Many of the questions raised were in the realm of confidential-administrative decisions, and the documented opinions rendered by the Committee supported the Food and Drug Administration in decisions and regulations which were most beneficial to the public.

More than twenty inquiries concerning drug treatment of allergic disease were referred to and answered by our Committee during the past year. Investigation of claims concerning several advertised products of either doubtful or unproved merit is under way.

Officers, Members, and Collaborators.—Murray Albert, Clarence Bernstein, Theodore B. Bernstein, Joseph Besser, Earl B. Brown, Seymour Crepea, Alex S. Friedlaender, Harold Friedman, Frank T. Joyce, Edmund Keeney, Saul Malkiel, Dean A. Moffat, Milton Mosko, Samuel Rynes, Irving W. Schiller, Sheppard Siegal, Aaron Spielman, Bert Schoenkerman, and Walter Winkenwerder.

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IV. COMMITTEE ON FOOD ALLERGY

The activities of the Committee on Food Allergy continued and broadened a program which has grown to its present scope during a period of several years.

1. Regional collecting stations for the reaginic serum bank were established in Chicago, Los Angeles, Wilkes-Barre, and Seattle, the latter under the direction of Dr. Norman Clein. Additional centers are under consideration.

2. The reaginic serum bank continues to store serum obtained from cooperating members. A form is being prepared for distribution among scientists who some day may require service from this agency. The form describes the function of the bank and lists the sera on hand: it thus acts

as a perpetual inventory and appraises the members of the Research Council and of the American Academy of Allergy of both our needs and our supplies. We plan to encourage publicity for this program through periodic insertions in the JOURNAL OF ALLERGY and through more active participation by the regional representatives.

3. The study of the question as to whether a common antigen exists in the fish family was initiated but not completed. It was decided to use the following list of fish for direct and indirect testing: shark, brook trout, goldfish, halibut, tuna, sturgeon, cod, and bluefish. Extracts of these fish were prepared by Margaret Strauss-Ballard. The possible antigenicity of cod-liver oil and of fish odors is also under consideration.

4. The study of the antigenicity of fresh fruit has progressed slowly. Steps have been taken to intensify the work by enlisting the aid of other investigators. The most urgent phase of this project is the procurement of the serum necessary for passive-transfer tests.

5. It was decided that the secretary of each subcommittee should prepare a written outline of experimental procedures under his jurisdiction. The secretary can thus be appraised of the activity of each subcommittee and have at all times a record of the projects as well as the individual assignments.

Officers, Members, and Collaborators.—Giacomo R. Ancona (fresh fruits), Katherine L. Bowman (reaginic bank), Norman Clein (reaginic bank), Sheldon G. Cohen (reaginic bank); Mary C. Johnson (allergenic extracts), Eloise W. Kailin (fish), Irwin C. Schumacher (fresh fruits), Bernard B. Siegel (allergenic extracts and reaginic bank), Margaret Strauss-Ballard (allergenic extracts), Abram M. Targow (reaginic bank), and Richard D. Wiseman (fish).

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V. SOME REMARKS ABOUT THE PRESENT AND FUTURE OF THE RESEARCH COUNCIL BY THE OUTGOING SECRETARY

During the past three years, the Research Council has moved toward its objectives: (1) to encourage discussions and disseminate information about the state of allergy and (2) to carry out group research. Individual research is not a concern of the Research Council.

The potential of the Research Council depends on (1) its administrative effectiveness, (2) the selection of suitable projects, and (3) the men and women who devote their efforts to its growth and development.

Administratively, the Research Council is decentralized. Its committees function as independent units. Occasionally, care has to be taken that the functions of individual committees do not overlap; increasing interest, for instance, in the standardization of techniques and interpretation of skin tests makes it necessary to decide where a subcommittee of this type belongs in principle and practice. Other problems arise; during the past year, the Research Council discussed the question of whether it might be practical to divide

committees into active and advisory units. The move is still under consideration; opinions range from Dr. George Blumstein's plea for a permanent Advisory Council of elder statesmen to the rather outspoken feeling of others that all committees must be active or else disbanded.

The projects of the Research Council are under constant scrutiny, not only by the organization itself, but apparently by the entire membership of the parent organization. In particular, the work of those sections which have functioned successfully for many years should not be taken for granted. It never occurred to me, for instance, that the outstanding work of our survey of pollen and molds might be open to criticism. Yet, it might be wise to accept a recent suggestion to report our findings in areas rather than in specific committees, since the towns and villages on our list may derive considerable economic advantage from our activities at the expense of others which might be equally suitable for hay fever sufferers but are not included in our roster of counting stations. Expanding research in allergy will most certainly make use of the specific capacity of the Research Council to test compounds and concepts in techniques which permit statistical evaluation.

In final analysis, the success of the Research Council is the success of the individual members who contribute their time and energy toward its activities. It is probably true that the world at large will measure the value of the Research Council by measuring its scientific accomplishments. I have come to feel, however, that the gathering of information—probably its most important objective—is only one of its functions. In a recent exchange of letters about the Research Council, I said: "It is, perhaps, natural to strive for perfection and to set one's goals as high as possible. It took me some time to learn that there are other rewards—for instance, how much satisfaction our members gain from participating in a group project, even if the project cannot possibly be classified as research." One of the members of the Executive Committee said, in a discussion of a recent interim report of the Research Council, that some of the most publicized activities of the Research Council could not possibly be considered scientific activities. I agree, and I also agree with the implied advice that it needs a rigid investigative yardstick for the projects which it adopts. Whatever the project, however, it establishes innumerable lines of communication between the Academy and its members who, although interested in the progress of allergy, might otherwise perhaps (for geographical reasons) be beyond our reach.

One of my most heartening personal experiences in 1956 was a generous, almost enthusiastic, response to an inquiry which served as a basis for the preparation of a treatise on legal problems which might arise in the field of allergy. I am greatly indebted to numerous contributors who offered their help when I enlisted the aid of the Research Council to gather a cross section of opinions.

Dr. Samuel M. Feinberg, who has guided the Committee on Air-borne Allergens for many years, has asked to be relieved from his position because

of the burden which the presidency of the International Association of Allergology has placed upon him. Dr. Lawrence J. Halpin has accepted our invitation to succeed him. Dr. Feinberg's counsel has been an inspiration to all of us. He has always been too generous with his time and, while we are reluctant to see him leave, we feel that no other decision was possible. With him go our regrets, our respect, and our affection. The Research Council wishes to extend its very best wishes to Dr. Feinberg and to his successor, Dr. Halpin, who, no stranger to us, will find himself surrounded by friends.

The Constitution and By-Laws of the Research Council limit the term of office of its secretary to three years. In other words, I have now finished the final year of my term. Dr. Kenneth P. Mathews of Ann Arbor, Michigan, who has been associated with Dr. John M. Sheldon for many years, has become the new secretary of the Research Council. While the limitation of tenure is, of course, healthy, I find it difficult to resign; I enjoyed my term of office very much. I am confident, however, that Dr. Mathews' willingness to assume the responsibility places the future of the Research Council into competent hands.

Respectfully submitted,

Max Samter, M.D., M.S.
Secretary.

REPORT OF THE COMMITTEE ON DRUGS OF THE RESEARCH COUNCIL OF THE AMERICAN ACADEMY OF ALLERGY, 1956-1957

THE Committee on Drugs is now composed of twenty active Members and Fellows of the Academy and two consulting pharmacologists. Its current roster is as follows:

Secretary:

Sidney Friedlaender, Detroit, Michigan.

Members:

Murray Albert, Brooklyn, New York.
Clarence Bernstein, Orlando, Florida.
Theodore B. Bernstein, Evanston, Illinois.
Joseph Besser, Philadelphia, Pennsylvania.
Earl B. Brown, New York, New York.
Seymour Crepea, Madison, Wisconsin.
Alex S. Friedlaender, Detroit, Michigan.
Harold Friedman, Cleveland, Ohio.
Frank T. Joyce, Denver, Colorado.
Edmund Keeney, La Jolla, California.
Saul Malkiel, Worcester, Massachusetts.
Dean A. Moffat, Salt Lake City, Utah.
Milton Mosko, Chicago, Illinois.
Samuel Rynes, Philadelphia, Pennsylvania.
Irving W. Schiller, Boston, Massachusetts.
Sheppard Siegal, New York, New York.
Aaron Spielman, New York, New York.
Bert Schoenkerman, Milwaukee, Wisconsin.
Walter Winkenwerder, Baltimore, Maryland.

Consultants:

Alfred Gilman, Ph.D., Professor of Pharmacology, Albert Einstein College of Medicine, Yeshiva University, New York, New York.
Harry W. Hays, Ph.D., Professor of Pharmacology, Wayne State University College of Medicine, Detroit, Michigan.

While the Committee's principal purpose has been the clinical evaluation of drugs, its activities during the past year have extended into other related spheres. Among these have been advisory services to the Food and Drug

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TABLE I. PREDNISONE AND PREDNISOLONE IN NONSEASONAL ALLERGIES
SUMMARY OF TREATMENT

CONDITION TREATED	PREDNISONE					PREDNISOLONE				
	TOTAL CASES	RESULTS				TOTAL CASES	RESULTS			
		EXCELLENT	GOOD	FAIR	POOR		EXCELLENT	GOOD	FAIR	POOR
Asthma	146	68	54	6	18	138	50	42	28	18
Rhinitis, nonseasonal	82	58	16	2	6	100	62	26	4	8
Urticaria, chronic	33	24	4	0	5	28	12	8	4	4
Urticaria, acute	8	8	0	0	0	8	8	0	0	0
Atopic eczema	22	10	4	4	4	18	12	4	1	2
Contact eczema	12	4	2	4	2	8	4	4	0	0

Administration; information to the medical profession, pharmaceutical industry, and the general public concerning the status of drugs applicable to the treatment of the allergic state; and investigation of unsubstantiated claims for the drug treatment of allergy.

CLINICAL EVALUATION OF DRUGS

Among the drugs submitted during the past year, five were selected for study from a number which met the criteria recommended by the Committee to the Research Council, and adopted by the Council.[1] These drugs were: prednisone and prednisolone, Histionex (phenyltoloxamine-resin complex), bronchodilator "JB-251," zinc-ACTH, and gamma globulin.

Prednisone and Prednisolone in Nonseasonal Allergies.—In a previous report of the Committee on Drugs, the use of two new steroids—prednisone (Meticorten) and prednisolone (Meticortelone)—submitted by the Schering Corporation, Bloomfield, New Jersey, was reported in considerable detail in relation to their effects on seasonal asthma and rhinitis due to pollen.[2] The present report completes the Committee's work with these analogues of cortisone and hydrocortisone and covers cases of nonseasonal allergy, such as chronic asthma, perennial allergic rhinitis, acute and chronic urticaria, atopic dermatitis, and contact dermatitis. The results of treatment are summarized in Table I. They amply confirm the previous findings that prednisone and prednisolone are of approximately equivalent clinical effectiveness, induce the same type of remission of symptoms as do the older steroids, and are effective in approximately one-fourth to one-fifth the dosage level. Most untoward effects generally associated with steroid therapy are noted at these therapeutic levels, although the tendency to retain salt and water is considerably lessened. On the other hand, a significant increase in the incidence of gastrointestinal side effects is noted with both prednisone and prednisolone. In common with our previously reported findings in pollinosis, prednisone and prednisolone must be continued in adequate dosage to maintain a remission of symptoms.

Histionex (Phenyltoloxamine-Resin Complex).—This preparation, submitted by R. J. Strasenburgh Company, Rochester, New York., is an antihistaminic-resin complex, designed to extend the effective duration of action of a single dose of antihistaminic drug. The antihistaminic employed in the combination, phenyltoloxamine (N, N-Dimethyl-2-[phenyl-o-toloxy] ethylamine), is one which has been in clinical use for several years.[3] The combination is based on the reasoning that prolonged clinical action might be possible if ionic equilibria were used to release the active drug through exchange with the ions of the intestinal tract at their commonly found concentrations. Laboratory data supplied by the manufacturer, utilizing simulated gastric and intestinal juices, indicated a sustained release of the antihistaminic over a prolonged period of time.

The primary purpose of the Committee's evaluation, which was carried out during the 1956 ragweed pollen season, was to determine whether a prolonged

clinical effect on hay fever symptoms was effected through the use of this antihistaminic-resin complex. The preparation was supplied in two strengths, containing 25 and 50 mg. of phenyltoloxamine, with matching control capsules of antihistaminic drug alone. The contents of each capsule were known to the participating investigators. Patients, however, were not aware of any difference between the identical-appearing capsules containing resin-complex and those containing a plain antihistaminic drug. The results of this study are summarized in Tables II and III. In 282 patients treated with the antihistaminic-resin complex, the clinical effectiveness in hay fever did not appear strikingly different from that observed in a group of 104 patients receiving the plain antihistaminic in the same dosage. Similarly, in 165 patients the duration of action of the antihistaminic-resin complex was approximately equivalent to that reported in a group of sixty-five patients receiving the plain antihistamine capsule.

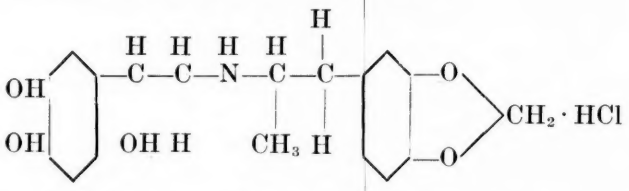
TABLE II. HISTIONEX STUDY (PHENYLTOLOXAMINE-RESIN COMPLEX)
CLINICAL EFFECTIVENESS OF HISTIONEX AND PHENYLTOLOXAMINE IN HAY FEVER

	HISTIONEX	PHENYLTOLOXAMINE
Total cases on each preparation	282	104
Results		
Excellent	29 (10.2%)	7 (6.7%)
Good	86 (30.4%)	47 (45.1%)
Fair	83 (29.4%)	19 (18.2%)
Poor	84 (29.7%)	31 (29.8%)

The duration of effect in the majority of patients comprising each group appeared to be less than eight hours. In forty-seven patients who noted some degree of effectiveness from both preparations, twenty-one felt that Histionex provided longer protection, two felt that the effect of phenyltoloxamine was longer, and twenty-four patients, or approximately one-half the entire group, could distinguish no difference between the two identical-appearing capsules.

These results do not indicate that the phenyltoloxamine-resin complex has a definitely prolonged clinical effect on hay fever symptoms as compared to phenyltoloxamine alone.

Bronchodilator "JB-251."—Our pharmacologic consultants felt that this N-substituted arterenol derivative, submitted by the Lakeside Laboratories, Inc., Milwaukee, Wisconsin, was worthy of careful study. JB-251 is alpha[(alpha-methyl-3,4-methylenedioxyphenylethylamino) methyl]—protocatechuyl alcohol hydrochloride, with the following structural formula:



It is a sympathomimetic amine with marked inhibitory action on smooth muscle, but with relatively little effect on the myocardium. A recent published report on its action in asthma was quite favorable.[4]

Three dosage forms were supplied to each participant: 2.5 mg. tablets for oral administration; 0.5 per cent solution for inhalation; and 1.0 mg. per cubic centimeter parenteral solution for intramuscular and subcutaneous injection.

TABLE III. HISTIONEX STUDY (PHENYLTOLOXAMINE-RESIN COMPLEX)
DURATION OF ACTION OF HISTIONEX AND PHENYLTOLOXAMINE IN HAY FEVER

	HISTIONEX	PHENYLTOLOXAMINE
Total cases on each preparation	165	65
Duration of action where present		
Over 12 hours	16 (9.9%)	10 (15.3%)
8 to 12 hours	17 (10.3%)	9 (13.8%)
4 to 8 hours	77 (46.6%)	28 (43.0%)
1 to 4 hours	55 (33.3%)	18 (27.6%)

While a final committee report on this drug is not available at this time, a total of fifty case reports submitted thus far indicates that JB-251 is an active bronchodilator when used by the oral or inhalation routes (Table IV). Reports

TABLE IV. BRONCHODILATOR DRUG JB-251
RESULTS IN ASTHMA

	2.5 MG. ORAL TABLETS	0.5% INHALATION SOLUTION
Total cases	40	10
Results		
Good	21	8
Fair	6	0
Unsatisfactory	13	2
Side Effects*		
None	19	10
Palpitation, tachycardia	19	0
Central nervous system stimulation	10	0
Gastrointestinal symptoms	1	0

*Nine patients experienced more than one side effect.

of cases in which the parenteral form was used are not yet available. Of forty asthmatic patients who received the 2.5 mg. oral tablets, twenty-one experienced good symptomatic relief, six had only fair results, and the effect in the remaining thirteen was termed unsatisfactory. This response was accompanied by a high incidence of side action, with twenty-one out of forty patients experiencing one or more untoward effects, namely, palpitation, tachycardia, and excessive central nervous system stimulation.

Of ten patients receiving the inhalation form, eight obtained good results, comparing favorably with other nebulized solutions, such as Epinephrine 1:100 and Isuprel 1:200. The results in only two patients were rated unsatisfactory. No side effects occurred in the smaller group using the 0.5 per cent nebulized solution of JB-251.

While it appears from the preliminary data that bronchodilator JB-251 may prove to be another addition to the symptomatic treatment of asthma, the frequency of side effects from the orally administered 2.5 mg. tablet would seem to call for a reduction in dosage or its combination with a barbiturate or other sedative.

Zinc-ACTH.—This preparation was submitted to the Committee for clinical evaluation in allergic states by Organon, Inc., Orange, New Jersey. The principal objective of the Committee's evaluation is to determine whether the duration of action of a single injection of the zinc preparation is greater than that of ACTH gel. Additional points for a comparison are the amounts of the zinc and the gel material necessary for suppression of symptoms and for maintenance therapy; also of interest are the comparative ease of administration and the occurrence or absence of local pain on injection.

Participants have been supplied with Organon's preparation of ACTH gel and with the newer zinc-ACTH for comparison. This study is still under way and the results will be presented upon completion.

Gamma Globulin Study.—Because of the current wide interest in the relationship of gamma globulin to infection, the Committee has decided to undertake a controlled study of the efficacy of gamma globulin administration in certain asthmatic states associated with bacterial infection. The American National Red Cross has agreed to supply the members of the Committee with sufficient materials to carry out this study. The project is planned as a long-term evaluation, extending over a period of at least one to two years. Reports of the progress of this work will be made in subsequent reports of the Committee.

ADVISORY SERVICES TO THE FOOD AND DRUG ADMINISTRATION

In 1956 the Committee was invited to serve as a liaison between the American Academy of Allergy and the Food and Drug Administration. During the course of the year a number of opinions were solicited from the Committee on problems confronting this governmental agency. These included advice regarding applications for over-the-counter sale of antiallergic drugs, labelling of new drugs, and incidence of allergic reactions to drugs in widespread use.

OTHER ACTIVITIES

The Committee on Drugs is repeatedly called upon to answer inquiries from the medical profession, pharmaceutical manufacturers, and the public concerning drugs applicable to the treatment of allergic disease. Some of these inquiries are referred from Academy headquarters, and others are forwarded by Members and Fellows of the Academy. More than twenty inquiries concerning the drug treatment of allergic disease were referred to the Committee and answered during the past year.

Investigation of unsubstantiated claims for the drug treatment of allergy appearing in medical journals and in the public press is also a necessary function of some branch of the American Academy of Allergy. These claims usually involve unknown preparations or drugs which have no previous documented background of successful use in allergic disease. Two requests for such investigation were directed to the Committee on Drugs during the past year.

One was received from the Food and Drug Administration, and involved a product called "Tyrodine" (manufactured by the Vitamin Quota Company, Los Angeles, California). This preparation, a mixture of tyrosine, pyridoxine,

and niacinamide, was advertised as an effective treatment for allergic states. The manufacturer claimed that tyrosine, as a precursor of epinephrine, when given orally, is converted to epinephrine which can diminish or prevent many of the pharmacologic effects of histamine produced in response to antigen stimulus. Pyridoxine and niacinamide are claimed to aid in this conversion of tyrosine to epinephrine. The opinion which Committee members and other Fellows of the Academy transmitted to the Food and Drug Administration was that there existed no scientific substantiation for this concept. Two important considerations were called to their attention: (1) there is more tyrosine available in foods than in the pharmaceutical preparations offered, and (2) enzyme inhibition of epinephrine has not prevented hypersensitivity responses in animals.[5]

Another request for investigation related to a preparation called "Anergex, a special extract of *Toxicodendron quercifolia,*" manufactured by the Mulford Colloid Laboratories, Philadelphia, Pennsylvania. Two recent publications, in addition to the manufacturer's advertising, claim extremely favorable results with this material in treatment of such conditions as asthma, allergic rhinitis, hay fever, drug reactions, eczema, and miscellaneous dermatologic lesions.[6] Neither the author nor the manufacturer is able to offer any scientific explanation of how the material works in producing its effect. The manufacturer has been invited to submit the preparation to the Committee for a controlled clinical evaluation, in order to determine whether it has the merit claimed. It is anticipated that the study will be carried out in the near future with the cooperation of the pharmaceutical manufacturer.

SUMMARY

The activities of the Committee on Drugs during the past year have included the clinical evaluation of prednisone (Meticorten) and prednisolone (Meticortelone), phenyltoloxamine-resin complex (Histionex), bronchodilator JB-251, zinc-ACTH, and gamma globulin. In addition, the Committee has furnished advisory services to the Food and Drug Administration, physicians, pharmaceutical manufacturers, and the public regarding drugs used in the treatment of allergy, as well as assistance in the investigation of unsubstantiated claims for drug treatment of allergic states.

Respectfully submitted,

Sidney Friedlaender, M.D., Secretary,
Committee on Drugs of the Research Council,
American Academy of Allergy.

REFERENCES

1. Report of the Research Council of the American Academy of Allergy, J. ALLERGY 27: 273, 1956.
2. Report of the Committee on Drugs of the Research Council, American Academy of Allergy, J. ALLERGY 27: 282, 1956.
3. Seyler, L. E., and Simon, S. W.: Bristamine, a New Antihistaminic Drug, J. ALLERGY 24: 261, 1953.

4. Itkin, I. H., Burrage, W. S., and Irwin, J. W.: Alpha (alpha-methyl-3,4-methylenedioxy-phenylethylamino), Methyl-Protocatechuyl Alcohol Hydrochloride in Bronchial Asthma, J. ALLERGY 27: 359, 1956.
5. Rehbun, J., Feinberg, S. M., and Zeller, E. A.: The Effect of Some Sympathicomimetic Amines and Enzyme Blocking Agents on Asthma in the Guinea Pig Induced by Antigens or Histamine Aerosols, J. ALLERGY 25: 440, 1954.
6. Rovito, J.: Non-Specific Therapy in Allergy: A Preliminary Report, Clin. Med. 2: 1009, 1955.
Rovito, J.: Non-Specific Therapy in Allergy: A Further Report, Am. Pract. & Digest Treat. 7: 1447, 1956.

Announcements

Workshop in Hypnosis

Long Island University Graduate School, in collaboration with the Institute for Research in Hypnosis, announces a workshop in hypnosis to be held on Oct. 23, 24, and 25, 1957, at the New York Academy of Sciences, 2 East 63rd St., New York City.

The workshop is planned to serve as an introduction into the nature of hypnosis and its psychodynamic and physiologic foundations, as well as clinical management in medicine, psychology, and dentistry.

For registration and particulars, write to The Institute for Research in Hypnosis, 200 West 57th St., New York 19, New York.

Allergy Society of the District of Columbia

The founding meeting of the Allergy Society of the District of Columbia was held on May 22, 1957. The following officers were elected:

President: Dr. Alvin Seltzer
Vice-president: Dr. Ellis April
Secretary-Treasurer: Dr. Marvin Fuchs

Appointed to the Executive Committee were Drs. Harry S. Bernton and William A. Howard. Organizations interested in affiliation and prospective applicants for membership may obtain information from the secretary, Dr. Marvin Fuchs, 5315 Connecticut Ave., N. W., Washington 15, D. C.

New Jersey Allergy Society

The newly elected officers of the New Jersey Allergy Society for 1957-58 are:

President: Dr. Harry Hershey, Jersey City, New Jersey
Vice-president: Dr. Frank H. Feldman, Newark, New Jersey
Secretary: Dr. Joseph Jehl, Clifton, New Jersey
Treasurer: Dr. Francis Pflum, Asbury Park, New Jersey

Books Received

Practical Otolaryngology. By Gervais Ward McAuliffe, M.D., F.A.C.S., F.I.C.S., Associate Clinical Professor of Otolaryngology, Cornell University Medical College; Attending Otolaryngologist, New York Hospital. 320 pages, with illustrations. New York, 1957, Landsberger Medical Books, Inc. Price, $7.00.

Ciba Foundation Colloquia on Endocrinology. Volume 10: Regulation and Mode of Action of Thyroid Hormones. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Elaine C. P. Millar, A.H.-W.C., A.R.I.C. 311 pages, with 114 illustrations. Boston, 1957, Little, Brown & Company. Price, $8.50.

Ciba Foundation Symposium on the Chemistry and Biology of Purines. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Cecilia M. O'Connor, B.Sc. 327 pages, with 124 illustrations and structural formulae. Boston, 1957, Little, Brown & Company. Price, $9.00.